JAN 7 1923
LIBRARY

MODIFIED LYMPH NODES FROM DOGS WITH A KNOWN HISTORY OF IRRADIATION, INCLUDING A NOTE ON 'GLOBULE' LEUCOCYTE FORMATION

ALDEN B. DAWSON
Department of Biology, New York University

FIVE PLATES (THIRTEEN FIGURES)

The material on which this study is based was obtained from irradiated dogs which were used by Ivy, McCarthy, and Orndoff ('24) in a study of the effect of Roentgen rays on secretory activity. In all three animals the area of exposure was the right lower abdomen. While at the time the tissue was obtained interest centered mainly on the histological changes produced in the small intestine (Dawson, '26), two lymph nodes, one from the pylorus and the other selected at random from the mesentery, were removed from each animal. The lymph nodes were sectioned, but received very little study until attention was focused on them by the recent studies of Jordan ('26, a, b, c). All the nodes give evidence of erythropoietic activity and exhibit modifications very similar to those described by Jordan.

The exponents of the monophyletic theory of blood-cell origin consider the small lymphocyte to be a hemocytoblast in a resting condition which can, as a result of certain stimuli, hypertrophy and become a large lymphoid cell or hemocytoblast and give rise to myelocytes, erythroblasts, and megakaryocytes. It is not the purpose of this paper, however, to present the x-ray as a specific agent which directly stimulates the lymphocyte into erythropoietic activity, but rather to present evidence which indicates that the effect of irradiation is indirect, producing, within the node, conditions favor-

THE ANATOMICAL RECORD, VOL. 36, NO. 1
JULY, 1927

able to the transformation of the lymphocytes into erythrocytes.

Latta ('21) has shown that the fate of the lymphocytes in the primary Peyer's patches of young rabbits is dependent on the vascular supply of that area. The lymphopoietic process is, apparently, in some way initiated by the presence of abundant lymphatic vessels, and a diffuse lymphatic tissue is produced. If the blood supply at any certain point is good, the lymphocytes multiply rapidly and become aggregated as dense nodular tissue about the blood vessels. The subsequent differentiation of the lymphocytes, he believes, is dependent upon the closeness of their association with the blood vessels, the slowness of the current of the blood stream, and upon the thinness of the vascular walls. When all these conditions are at their optimum, erythropoiesis occurs. If they are present, but in a lesser degree, granulopoiesis results.

In 1923, Mottram made an intensive study of the iliac lymph gland of the rat after exposure of the entire animal to radiations from radium. In the medulla of the node he distinguished areas of cells (plasmoidocytes of Maximow) grouped about the blood vessels and separated by lymphatic channels. According to Mottram, the cells of these areas closely resemble cells in the spleen which give rise to red cells, and very occasionally he observed them performing the same function in the iliac gland. Mottram believes that the plasmoidocytes, by division, may give rise to small lymphocytes which enter the circulation mainly by way of the efferent lymphatics, but they may also pass directly through the vascular wall into the blood vessels.

More recently, Jordan ('26, a, b, c) and Jordan and Looper ('27) have added materially to the evidences of erythropoietic activity in the mammalian lymph node. In certain nodes ('26 b) granulocytopoiesis was also observed, but the special granulocytes were differentiated in part from reticular cells in the form of hemohistioblasts. The transformation of lymph nodes, according to Jordan, is correlated with the atrophy and disjunction of the lymphatics. In the nodes

obtained from the rabbit and dog the disturbance of the lymphatic circulation was interpreted in terms of an initial disjunction of the afferent lymphatics, followed, after an interval permitting almost complete drainage of the medullary sinuses and the deeper portions of the cortex, by atrophy and disconnection of the efferent lymphatics. Subsequently, the remaining cortical lymphocytes largely degenerate, while the medullary cordal lymphocytes differentiate into erythrocytes which secondarily enter newly developed vessels within the cord, the axial venules. The three human lymph nodes show a similar modification of function apparently as the result of a reverse process, namely, a primary atrophy and disjunction of the efferent lymphatics. In these nodes the central drainage by way of the efferent lymphatics would not occur. The lymphocytes of the now closed sinuses would be the first to differentiate into erythroblasts, since, in general, they represent older stages and would precede the younger lymphocytes of the nodules and cords in the process of erythrocytopoiesis. In addition to the loss of the lymphatics, Jordan also finds evidences of a sluggish circulation within the blood vessels of the node.

Definite evidence of granulocytopoietic activity within aggregated lymphoid tissue has also been obtained by Bloom ('27). In a series of experiments in which guinea-pigs were sensitized with chicken erythrocytes and then thrown into anaphylactic shock, two weeks later, by a second injection of the same antigen, he found in the germinal centers of the nodules of the lymph nodes and spleens of some animals a highly demonstrative picture of granulocytopoietic transformation of small lymphocytes.

The effects of stagnation of circulation have also been noted by Maximow ('24) in the experimental production of 'bone marrow' in the rabbit's kidney after ligation of its blood vessels.

It does not appear necessary or feasible to make a complete survey of the literature dealing with the conflicting evidence and views of various authors regarding hematopoiesis. The

evidence in favor of the monophyletic theory is adequately covered by Maximow ('24, '27), Jordan ('26 a, b), and Bloom ('27).

MATERIAL

As previously noted, the material described in this paper was obtained from three dogs which had been exposed to x-rays. Six nodes in all were obtained, one pyloric and one mesenteric from each animal.

Dog 1. The roentgen-rays dose given was: 110 kilovolt minutes, 5 ma., 30 cm. focal skin distance, 6 cm. diameter portal, 1 mm. aluminum filter for thirty-seven minutes, delivered over the right lower abdomen. On the fourteenth day, epilation and vesiculation of the skin appeared. The dog lost weight rapidly. On the twenty-second day a complete count showed the blood to be normal. The dog died on the twenty-third day. Necropsy revealed two duodenal ulcers and early gangrene in the anterior ileum. The lymph glands of the abdominal cavity were soft.

Dogs 2 and 3 received equal doses of x-rays: 20 kilovolt minutes, 5 ma., 50 cm. focal skin distance, 11 $\times$ 21 cm. portal, 0.75 mm. copper and 1 mm. aluminum filter, for ninety minutes, over the entire abdomen from the right side. After recovery from the effects of the irradiation, dog 2 remained in good condition and was killed 242 days after the exposure. The blood was normal in every respect.

Dog 3, however, never completely recovered from the effects of the irradiation and lost weight rapidly and died 102 days after exposure. The cause of death was not known. This dog showed marked anemia with two and one-half million reds present. The blood clotted very rapidly. The dose given to dog 1 was a dog's erythema, equal to two and one-half human erythemas. That given dogs 2 and 3 was only 75 per cent of a human erythema.

All material was fixed in Helly's fluid and stained with iron hematoxylin, hematoxylin and eosin, or eosin and methylene blue. For some unknown reason, the fixation of the nodes

from dog 3 was faulty, but the conditions appear to be essentially similar to those observed in the tissue from dog 2. Accordingly, the description of the material will be based on the four nodes from dogs 1 and 2.

DESCRIPTION OF MATERIAL

Dog 1. The description logically begins with the pyloric and mesenteric nodes from this animal. Both nodes represent a rather late stage in the series of regressive changes which Jordan ('26 a, b) has outlined, and resemble very closely the submaxillary node of a rabbit described by him ('26 a). Since these nodes are similar in most respects, they will not be described separately, but minor differences between them will be indicated as the description progresses. The shape and proportions of the mesenteric node are adequately shown in figure 1. A hilum is present at one pole, but the blood vessels leave and enter over a considerable area at this pole. The pyloric node is bean-shaped with a distinct hilum. No afferent or efferent lymphatics could be distinguished in either node.

In both nodes the cortex is greatly reduced and is interrupted at many points by modified tissue which extends to the peripheral sinus. The atypical cortical tissue is not sharply marked off from the medulla and no lymph nodules are visible. The medulla consists of a diffuse lymphatic tissue in which slender irregular cords can be distinguished.

The cortex, where present, consists of a dense lymphoid tissue, with small lymphocytes predominating, but in many areas all stages of erythrocytopoiesis, from normoblast to red blood corpuscle, can be distinguished (fig. 5). The more typical and compact areas of cortical tissue merge with the more diffuse medullary tissue and are also interrupted by peripheral extensions of medulla-like tissue. The differentiating erythrocytes are usually found in the vicinity of thin-walled sinusoidal blood vessels which probably represent extensions of the axial vessels of the medullary cords, which

will be discussed later. The modified lymphocytes of these areas stain lightly, due to the vesicular character of their nuclei and chromophobic reaction of their cytoplasm. However, little evidence of degenerative changes within the lymphocytes can be found and mitotic figures are absent. However, both nodes are characterized by the presence of large numbers of mononucleated phagocytes (figs. 5, 9). These macrophages are equally distributed through the atypical cortex and the medullary cords. A few are also found within the sinuses. They are usually spherical in form with an eccentric nucleus and contain large amounts of ingested material. They usually appear yellow or brown in color, but occasionally the irregular cytoplasmic inclusions stain bright red with eosin. The chief material ingested appears to be red blood cells and the characteristic color of the cells is presumably due to hemoglobin débris. The origin of these cells is problematical. Jordan ('26 b) believes that they are modified stromal cells, but it seems possible that they may also represent modified lymphocytes (monocytes). At least they are quite unlike other large cells, found within the sinuses of these nodes, which can readily be demonstrated to be of stromal origin (figs. 6, 7, 11, 12).

The modification of the cortex is apparently due to erythroblastic differentiation of lymphocytes, especially along its medullary border, followed by the drainage of the differentiated lymphocytes by way of vascular extensions from the medulla. Since there was little lymphocytic proliferation within the cortex, the processes of erythrocytopoiesis and drainage resulted in the local exhaustion of this zone and permitted the medulla-like tissue to extend to the periphery. The differentiated red blood corpuscles apparently have the physiological status of foreign bodies and stimulate the differentiation of macrophages.

The medulla consists of large irregular sinuses separated by slender irregular cords of compact lymphoid tissue. There is no means of determining the relationship of these sinuses to those normally present in a lymph node. Presumably, they

represent the original sinuses enlarged by the reduction of the medullary cords and extended toward the periphery by the modification of the cortex. The reticular cells of the stroma exhibit considerable modification. The fixed cells either form a wide-meshed reticulum within the sinuses (figs. 6, 7) or become arranged on the surface of the cords to form a distinct membrane almost identical with endothelium (fig. 13). Many of the fixed cells appear swollen and are highly vacuolated. Other cells probably representing free reticular cells are also present. These also possess a vacuolated protoplasm and tend to be spherical in outline (figs. 6, 7, 11, 12, *a*). Their nuclear pattern is variable, some being mononucleate, others polymorphonucleate, and others binucleate. Occasionally they exhibit phagocytic activity (figs. 7, 11). Many closely resemble plasma cells (figs. 7, c, 12, b). Besides the reticular cells, the sinuses contain erythroplastids, erythroblasts at various stages of differentiation, scattered normal lymphocytes (fig. 7), and occasional mononucleated phagocytes similar to those found in greater abundance in the cortex and the medullary cords. This description of the stroma and parenchyma of the medullary sinuses applies equally well to the peripheral sinus.

The compact lymphoid tissues of the medullary cords of these nodes exhibit the same modifications as that of the cortex. Many cords contain an axial blood vessel (axial venule of Jordan and Looper, '27) as shown in figure 13. Some lack the axial venule and in others the venules are in process of obliteration (fig. 7) through the interruption of the endothelium and the invasion of the differentiating erythrocytes. In some areas practically all blood cells have disappeared from the sinuses and cords (fig. 7), due to the loss of differentiated corpuscles either by drainage into the axial venules or by their destruction through phagocytosis.

Most of the cords consist of normoblasts and erythroblasts (fig. 13), with occasional completely differentiated red blood corpuscles. The cytoplasm of the differentiating erythrocytes is usually colored red in the hematoxylin-eosin technique, but

in some cells it still exhibits a slight basophilic reaction as well. The change from the basophilic to the acidophilic reaction and associated nuclear changes are indications that the lymphocytes are elaborating hemoglobin, but this cannot be regarded as a final criterion, since under many experimental conditions, such as exposure to irradiation, the nuclear pattern and the staining reactions of cytoplasm may be modified. These definite indications of lymphocyte transformation, however, are further substantiated by the presence within their midst of fully differentiated red blood corpuscles. The staining reactions referred to, and the changes in nuclear size and pattern are usually accepted as sufficient criteria for the identification of blood elements. There is, unfortunately, no satisfactory specific elective stain or other micro-chemical test for hemoglobin. Atypical erythrocytes of problematical significance are occasionally encountered (fig. 13, d, e), but no granulocytes occur anywhere within these lymph nodes. Other special cells, apparently similar to the globule leucocytes (Schollenleukozyten) described by Weill ('19) as occurring in the alimentary epithelium of the dog, cat, and mouse, are found scattered through the modified cortex and cords of these nodes. Only a few occur in the mesenteric node, but they are quite plentiful in the pyloric node. These cells will be discussed in detail below.

Dog 2. The pyloric and mesenteric nodes of this animal are in approximately the same stage of transformation. A complete series of sections of the pyloric node was prepared. The mesenteric node was much larger and the entire node was not sectioned. The pyloric node will form the basis of the following description. It represents a critical stage in the obliteration of the lymphatic supply and, on account of the completeness of the serial sections, the condition of the lymphatic connections can be accurately determined.

Both nodes are essentially alike and are at much earlier stages in the process of regression than those from dog 1. They are comparable to the two nodes from a dog described by Jordan ('26 b). A definite cortex is present and shows

distinct nodules with germinal centers (fig. 3). The medullary border of the cortex exhibits considerable modification and in appearance suggests the spleen (figs. 3, 4, *tr.zone*). The medulla is essentially like that of the nodes from dog 1, but does not show such advanced stages of erythrogenic activity.

Both nodes possess a distinct peripheral sinus, and in the pyloric node five patent afferent lymphatics can be traced into it. All of these lymph vessels enter at one pole and the perinodular and peripheral portions of the medullary sinuses in the vicinity are greatly distended, indicating that the drainage from the node had previously been obliterated (fig. 2). No efferent lymphatics were found. In this node, at least, lymph stasis was produced by the primary atrophy and disjunction of the efferent vessels, and this was probably followed by the atrophy of the afferent lymphatics over the major portion of the node, leaving a few vessels at one pole still patent.

The cortex also presents significant changes. Within the nodules and in their immediate vicinity the lymphocytes are of the normal type (fig. 4, *e*). Toward the periphery and adjoining the peripheral sinus the lymphocytes are larger and stain more lightly (fig. 4, *d*). This is due to the relative increase in the volume of the cytoplasm and the greater vesiculation of the nuclei of these cells. This change in the staining reaction is more pronounced toward the medulla (fig. 4, *f*) and is due to the accentuation of the changes already noted in the lymphocytes of the peripheral zone. Between this inner zone and the typical medulla is a transitional zone which resembles splenic pulp in many respects. It is composed of denser lymphatic cords of rather indefinite outline and looser intercordal tissue composed of differentiating erythroblasts and red blood cells (fig. 8). The intercordal tissue probably represents sinuses filled with differentiating erythrocytes. Whether these represent perinodular sinuses filled with differentiating cells or represent areas of differentiating lymphocytes which have been more or less isolated from the normal lymphocytes by a secondary formation of an

endothelioid membrane from reticular cells it is impossible to determine from a study of this node. These areas of erythrogenic activity are pierced by straight thin-walled blood vessels (figs. 3, 4, *b.v.*) which represent extensions of the vascular tree of the node. Some appear to be continuous with the axial venules of the medullary cords, others cannot be traced to these vessels, but apparently arise directly from the veins of the node.

The straight veins appear to be temporary structures, their endothelium eventually becoming continuous distally with the reticulum-endothelium of the blood-laden sinuses. In this manner extravascularly differentiated red blood cells are introduced into the circulation. Subsequently, these hemal connections are probably obliterated and the empty sinuses may be occupied later by scattered lymphocytes which migrate from the adjoining tissue. In this fashion the cortical tissue would suffer continuous erosion, and eventually the medulla-like tissue would extend to the peripheral sinus—a condition which has already been described in the nodes from dog 1.

The medullary tissue of both nodes from dog 2 is relatively more dense than that of the nodes from dog 1. The cords, however, are relatively narrow and the predominating cell is the normoblast, but typical lymphocytes and erythrocytes are also present. Many of the cords possess an axial venule which, as already noted, can in some instances be traced to the transitional zone of the cortex where erythrogenesis is taking place rapidly. The medullary sinuses, especially of the pyloric node, are crowded with differentiating lymphocytes, normoblasts, erythroblasts, and red blood cells. Toward the cortex the medullary sinuses are in many cases continuous with the sinuses of the transitional zone, but the number of red blood cells within the sinuses of the transitional zone is much greater. The dense appearance of the medulla is largely due to the number of cells within the sinuses of this region. Mononuclear phagocytes are practically absent from all parts of the nodes and little destruction of red blood cells is taking place. In many cords the endothelium of the central blood

vessels has suffered disruption, the lymphocytes of the cords have practically disappeared and the pericordal reticulum-endothelium is no longer typically developed. It appears, accordingly, that the medullary sinuses as well as the sinuses of the transitional zone of the cortex may be secondarily placed in direct communication with the lumina of the axial venules permitting the differentiated erythroblasts to enter the hemal circulation. The peripheral sinus, however, is practically empty and contains few cells other than reticular elements. Thus the medullary and transitional zones of these nodes have the relation to the hemal circulation which are found in hemal nodes, but the more peripheral cortex, characterized by lymph nodules and 'germinal centers' and separated from the capsule by a definite peripheral sinus, still retains the typical relations and appearance of the cortical portion of a normal lymph node.

'GLOBULE' LEUCOCYTES

As already noted, the so-called globule leucocytes are found rather uniformly distributed throughout the cortex and medullary cords of both lymph nodes from dog 1, the pyloric node containing relatively the greater number of cells. These cells, when typically developed, are characterized by the presence of a large number of acidophilic inclusions (fig. 10). These eosinophile cells have usually been described as occurring in the omentum and in the mucosa of the alimentary canal. Maximow ('24) summarizes the various views regarding these cells as follows: "The eosinophile cells, in other places, for instance in the omentum, described by Dominici ('09) and Pappenheim ('09) are possibly not true eosinophile leucocytes nor myelocytes at all, but lymphoid cells, elaborating in their protoplasm a peculiar acidophilic substance in the form of large granules or even lumps of uneven size and shape. They might be identical with similar cells described by Weill ('19) as 'Schollenleuozyten' in the intestinal mucosa, or with plasma cells, elaborating acidophilic inclusions (Dubreuil and Favre, '21) or with lymphoid cells

with acidophilous granules, found by Solucha ('08) in the loose connective tissue of the adult chick.'' More recently, similar cells have been described by Keasbey ('23) in the gastric mucosa (abomasum) of the sheep. In this instance they were usually located exclusively within the epithelium. Keasbey was impressed by the great similarity which the globules displayed, both in general morphology and staining reaction, to erythrocytic material, and this led her to employ a number of microchemical tests, all of which proved confirmatory of the erythrocytic nature of the globules. She considers the origin of the globule leucocytes as highly problematical, rejecting the interpretation that the globules may be secretory elements. This has been discussed by the author in another publication (Dawson, '27). She also feels that the evidence against the view that these globules are phagocytosed particles of erythrocytes rules out such an explanation. She believes that the globule leucocyte is in some way connected with a slow degeneration of tissues, that occurs subsequent to a lymphoid infiltration, and, in conjunction with the formation of lymphoid nodules in the gastric mucosa. She also noted evidences of stasis and capillary degeneration. Weill ('19) regards these cells as of lymphocytic origin and the globules (Schollen) as endogenous secretion granules, although he discusses other possible interpretations of this peculiar cell form. Clara ('26) has made an intensive study of these cells and their distribution throughout the digestive tract of a large number of birds, and concludes: ''Die Elemente stellen zweifellos Abkömmlinge von Wanderzellen dar, in deren Protoplasma aus noch nicht näher bekannten Gründen grobe Einlagerungen aufgetreten sind.'' No cells of this type were mentioned by Jordan ('26 a, b) unless the cell shown in figure 5, *d* ('25 a) can be so interpreted.

In the lymph nodes of the dog in which the globule leucocytes occur there is also abundant evidence that phagocytosis of red blood cells by monocytes is taking place. The mononuclear phagocytes are of the same size as the globule leucocytes and the position and pattern of their nuclei are also

similar (figs. 9, 10). Accordingly, these cells can be differentiated solely by the staining reaction of their cytoplasm and the nature and staining of their inclusions. Within the phagocytes the fragmented red blood cells are usually irregular in outline and many have a yellow or brown color due to the changes in their hemoglobin content. In the globule leucocytes the inclusions are usually of definite form and stain uniformly, but they may vary considerably in size. The staining reactions to eosin and to iron hematoxylin are the same as that of erythrocytic material. No other microchemical tests were made on these cells, but they are obviously similar to the cells tested by Keasbey and appear identical with similar cells in the intestinal mucosa of the dog (Weill, '19). There is little to suggest that the globules are derived from ingested erythrocytes, although their close association with phagocytic cells of similar size and shape favors such an interpretation. An intensive study of the pyloric node revealed several cells, comparable in general appearance and staining reactions to normoblasts, which contained a small number of globules (fig. 10 *a*). As the number of globules within the cell increases, the basophilic tint of cytoplasm is no longer evident and the cytoplasm is purely acidophilic, and in more completely differentiated cells the cytoplasm usually fails to stain. It is, accordingly, possible to find a complete series of transitional stages from unmodified lymphocytes to normoblast-like cells with a few globules to the fully differentiated globule leucocytes. The globules apparently have an endogenous origin, but their functional significance is not evident. This special leucocyte appears to represent a blood element which possesses features characteristic of both erythrocytes and leucocytes. If the microchemical tests employed by Keasbey are valid, they have the ability to elaborate hemoglobin which is not diffusely distributed through the cytoplasm, but restricted to the globules, and they also retain their nuclei. On the other hand, they are capable of migrating through the tissues, as is evidenced by their frequent occurrence within the epithelium of the digestive tract.

DISCUSSION

The theoretical and practical aspects of the erythrocytogenic capacity of mammalian lymph nodes have been fully discussed by Jordan ('26 a, b), and need not be repeated in this paper. Accordingly, this discussion will be concerned mainly in correlating the conditions presented by the six nodes herein described with the six nodes (one from a rabbit, two from a dog, and three from a man) on which Jordan's descriptions were based. All the nodes give evidence of profound changes, which are mostly of a regressive nature and which would probably result in the destruction of the node. Jordan has suggested that the so-called hemal node may be interpreted as a stage in the involution of a transitory lymph node, due to the differentiation of the lymphocytes, isolated from the lymphatics, into red cells, first in the sinuses and later in the medullary cords.

Jordan believed that the series of regressive changes were initiated by the disruption of the lymphatics, and he postulated that in some cases the afferent lymphatics and in others the efferent lymphatics were the first to disappear. The ultimate fate of the node would not be affected by the reversal of the order of disruption of the lymphatics, but the appearance of the node while undergoing regression would be modified, since in the case with the efferent lymphatics patent after the afferent lymphatics had atrophied a 'central' drainage of lymphocytes from the sinuses would result. When the reverse was true, the sinuses would be well-filled with lymphocytes, and since these cells are older they would be the first to undergo erythrocytic transformation. It is difficult to determine the causes underlying the atrophy and disjunction of the lymphatics supplying a node, whether they are inherent in the life-history of the node or whether they are the result of more widespread systemic influences. Irradiation was regarded as a possible factor in the production of lymph stasis, but the recent observations of Jordan and Looper ('27) on the rabbit lead one to suspect that the atrophy

of the lymphatics following exposure to x-rays was perhaps a coincidence rather than a result. Jordan and Looper find that lymph nodes are normally more or less evanescent structures, especially those of the cervicosubmaxillary and subcutaneous groups, and that the intermediate stages of their repression are comparable to the modified lymph nodes described by Jordan and by the writer. Although the cause of lymphatic disruption still remains obscure, the order of disruption is well illustrated by the pyloric node from dog 2, in which five patent afferent vessels were found entering the peripheral sinus. This condition associated with the extreme distention of the sinuses in the vicinity of the afferent vessels indicates that the efferent lymph vessels must have previously suffered disruption. However, this order may not be maintained for every node, but as yet it is only in this one instance that any definite evidence has been obtained on the subject.

The nodes from dog 2 represent intermediate regressive stages in which the cortical area is still present, but its medullary border, designated in this paper as the transitional zone, shows striking evidences of erythrocytogenic activity. In the nodes from dog 1 the result of the progressive expansion of the medulla has resulted in almost complete obliteration of the cortex. The erythrocytes differentiated within the medullary sinuses, the sinuses of the transitional region, and the medullary cords are drained into the venous circulation after disruption of the capillary endothelium and the invasion of their lumina. In some instances the presence of erythrocytes stimulated the production of phagocytic giant cells, and Jordan ('26 c) thinks that the red blood cells of the sinuses are removed by this method instead of through the hemal circulation. In the rabbit's node Jordan ('26 a) and in the nodes of dog 2 phagocytic cells were not present. Moreover, in my preparations there is evidence that the erythrocytes of the sinuses as well as those of the cords leave the node through the blood vessels. It is difficult to understand why the production of phagocytes should occur in some cases and not in others.

The medullary cords of normal nodes usually lack a blood vessel, and the frequent occurrence of these axial vessels in the cords of modified lymph nodes indicates that they are the result of a secondary invasion of these regions. Jordan ('26 a) and Latta ('21) believe that the primary factor involved in stimulating erythrocytopoiesis is probably a transudation product of the blood plasma. Accordingly, the secondary vascularization would be the cause, and not the result of erythrocytopoiesis. If this be the correct interpretation, the cause of the secondary extension of the vascular tree is probably to be found in the condition of lymphatic stasis caused by the atrophy and disjunction of the lymphatics. Further than this we cannot trace the sequence of events unless, as has already been suggested, the exposure of the nodes to irradiation has been the direct cause of lymphatic stasis; but in all the nodes described by Jordan and Jordan and Looper there was nothing in the previous histories of the animals which could be suspected of initiating the atrophy of the lymphatics.

CONCLUSIONS

A study of the six lymph nodes from three dogs previously exposed to Roentgen rays confirms the earlier observations of Jordan on the erythrocytogenic capacity of mammalian lymph nodes and the stages of regression as outlined by him are also found in these nodes.

In one pyloric node definite evidence was obtained on the manner and order of lymphatic disjunction. In this case the efferent vessels were absent, but five patent afferent vessels were found at one pole, and the perinodular and peripheral portions of the medullary sinuses in the vicinity of the afferent vessels were greatly distended, also indicative of a primary blockade of the efferent flow.

The conclusions reached in this paper are, with one exception, in complete accord with those of Jordan. He believes that the lymphocytes trapped within the sinuses differentiate into erythrocytes, later disintegrate, and pass into

solution or are removed by giant cells or mononuclear phagocytes. There is some evidence in my preparations that the blood-filled sinuses may acquire temporary hemal connections which permit of drainage of the differentiated erythrocytes. The best evidence in favor of this view is found in the so-called transitional zone, a spleen-like tissue formed by the transformation of the medullary border of the cortex. Here straight thin-walled vessels appear to become continuous with the sinuses which are crowded with red blood cells. Drainage from the medullary sinus, however, does not appear to occur so rapidly, but awaits the local exhaustion of the vascularized medullary cords. The delayed drainage of these areas usually permits considerable phagocytosis to occur, but in two nodes studied, although there was an abundance of red blood cells in the sinuses, practically no phagocytosis was observed. Conclusive evidence on this point is lacking. Another conclusion also seems open to question, the one regarding the significance of the extension of the vascular tree of the node. Jordan thinks that the presence of the blood vessels is the initiating stimulus for erythrocytic differentiation. It seems equally possible that lymph stasis may be the factor initiating erythrocytopoiesis, and the extension of the hemal circulation occurs as a response to the presence of differentiating red blood cells in a non-vascularized area.

The cause of lymphatic stasis is unknown. It is suggested that in the animals exposed to irradiation the atrophy of the lymph vessels was due to the effects of the x-rays. But in the light of the many cases presented by Jordan in which there was nothing abnormal so far as is known in the life-history of the animals, the lymphatic atrophy found subsequent to irradiation may be regarded as a coincidence rather than a result. The cause of lymphatic atrophy, accordingly, may be due to local conditions within the node or to more widespread systemic conditions which modify the lymphatic connections.

Globule leucocytes ordinarily found within the epithelium and connective tissue of the mucosa of the digestive tract were

also found in two lymph nodes. Transitional stages from normal lymphocytes to normoblast-like cells with few globular inclusions to the completely differentiated special leucocytes were noted. It is suggested that these leucocytes represent cells which occupy an intermediate position between red blood cells and leucocytes, since the globules react chemically like erythrocytic material and the cells also possess the ability to migrate, as is indicated by their frequent occurrence within the epithelium of the alimentary tract.

LITERATURE CITED

Bloom, W. 1926 The hemopoietic potency of the small lymphocyte. Folia Haemat., Bd. 33, S. 122–131.

Clara, M. 1926 Beiträge zur Kenntnis des Vogeldarms. Teil VI. Das lymphoretikulare Gewebe im Darmröhre mit besonderer Berücksichtigung der leukozytären Zellen. Zeitschr. f. mikr.-anat. Forsch., Bd. 6, S. 256–350.

Dawson, A. B. 1926 A histological study of the response of the intestinal mucosa of the dog to irradiation, with special reference to giant cell formation. Jour. Exp. Zoöl., vol. 46, pp. 467–491.

——— 1927 The various elements in the epithelium of the fundic mucosa, with special reference to those cells whose entodermal origin is doubtful. Anat. Rec., vol. 35, pp. 99–108.

Dominici, H. 1909 De l'origine lymphatique ou amyéloide des polynuclaires ou leucocytes granuleux à noyau polymorphe. Folia Haemat., Bd. 8, S. 97–106.

Dubreuil, G., et M. Favre 1921 Cellules plasmatiques. Plasmazellen à granulations specifiques. Cellules à corps de Russell. Arch. d'Anat. Micr., T. 17, p. 303.

Ivy, A. C., J. E. McCarthy, and B. H. Orndoff 1924 The effect of exposure of abdominal and thoracic areas to Roentgen rays on gastric secretion; note of Roentgen cachexia. Jour. Am. Med. Assoc., vol. 83, pp. 1977–1983.

Jordan, H. E. 1926 a The transformation of lymphocytes into erythroblasts in a lymph node of a rabbit. Anat. Rec., vol. 32, pp. 369–393.

——— 1926 b The erythrocytogenic capacity of mammalian lymph nodes. Am. Jour. Anat., vol. 38, pp. 255–280.

——— 1926 c The significance of hemal nodes. Anat. Rec., vol. 34, p. 140 (abstract; the complete paper to appear in the Journal of Morphology and Physiology).

Jordan, H. E., and J. B. Looper 1927 The comparative histology of the lymph nodes of the rabbit. Anat. Rec., vol. 35, p. 16 (abstract).

Keasbey, L. E. 1923 On a new form of leucocyte (Schollenleukozyt, Weill) as found in the gastric mucosa of the sheep. Folia Haemat., Bd. 29, S. 155–171.

LATTA, J. S. 1921 The histogenesis of dense lymphatic tissue of the intestine (Lepus). A contribution to the knowledge of the development of lymphatic tissue and blood-cell formation. Am. Jour. Anat., vol. 29, pp. 159–212.

MAXIMOW, A. A. 1924 Relation of blood cells to connective tissues and endothelium. Physiol. Rev., vol. 4, pp. 533–563.

———— 1926 Über undifferenzierte Blutzellen und mesenchymale Keimlager im erwachsenen Organismus. Klin. Wochensehr., Berlin, Bd. 5, S. 2193–2199.

MOTTRAM, J. C. 1923 Some observations upon the histological changes in lymphatic glands following exposure to radium. Am. Jour. Med. Sci., vol. 165, pp. 469–479.

PAPPENHEIM, A. 1909 Zu vorstehender Mitteilung Dominici's. Folia Haemat., Bd. 8, S. 107.

SOLUCHA, N. 1908 On the cells of the connective tissue of the birds in normal conditions and in inflammation. St. Petersburg, Inaug. Dissert. (in Russian); reviewed, Folia Haemat., 1909, Bd. 8, S. 230.

WEILL, P. 1919 Ein Beitrag zur Beurteilung der Granulationen in Leukozyten. Über die leukozytären Elemente der Darm-Schleimhaut der Säugetiere. Arch. f. mikr. Anat., Bd. 93, S. 1–81.

EXPLANATION OF PLATES

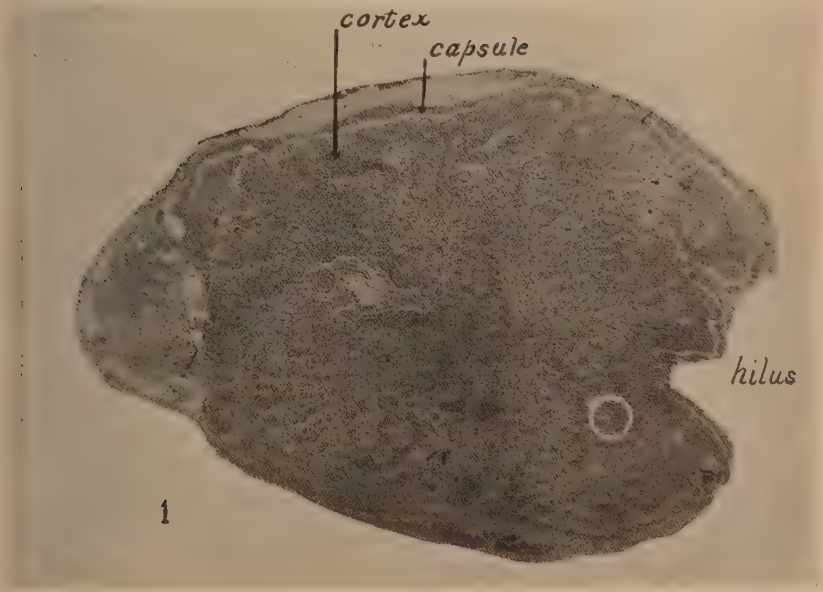
Figures 1 to 8 are microphotographs: 1 to 3 with a 35-mm. objective, 4 with a 16-mm. objective, and 5 to 8 with an oil-immersion objective. They are reproduced without any reduction in size. Figures 9 to 13 were outlined with a camera lucida and drawn at the same magnification.

PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section of the mesenteric node from dog 1, showing the gross internal arrangement. Note the remnant of the cortex and the slender irregular medullary cords. The peripheral sinus is present, but no trace of afferent or efferent lymphatics was found. An area included within the white circle is shown in figure 13.

2 A longitudinal section, showing one pole of the pyloric node from dog 2. This is a tangential section, and accordingly passes through the cortex only. A medial longitudinal section of this node is shown in figure 3. Two afferent lymphatics are shown entering the peripheral sinus. Owing to the obliteration of the efferent lymphatics, the peripheral and perinodular sinuses have been greatly distended.


cortex
capsule
hilus
1

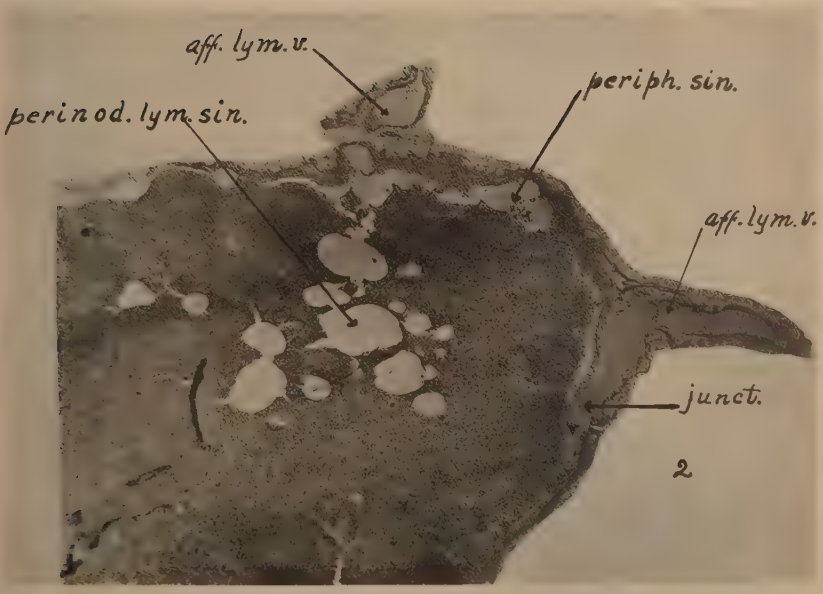
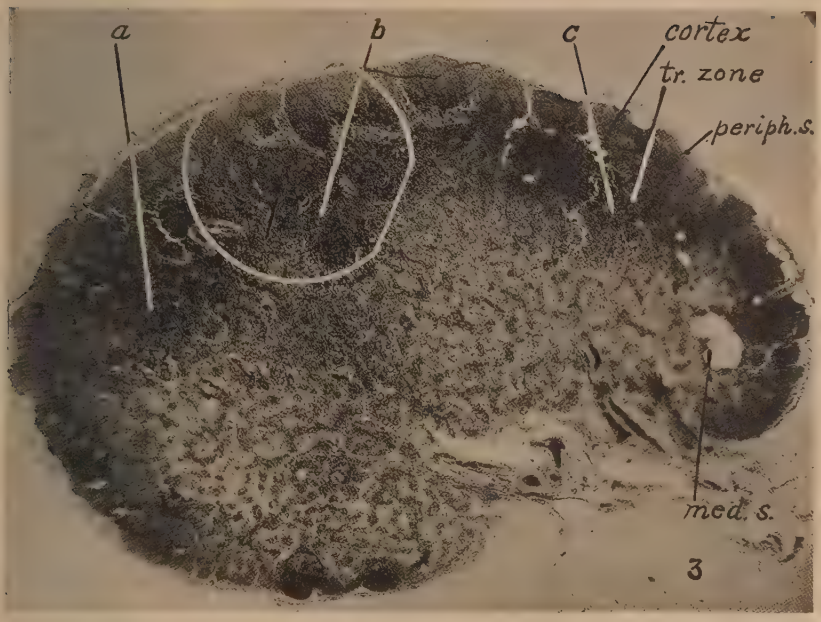

aff. lym. v.
periph. sin.
perinod. lym. sin.
aff. lym. v.
junct.
2

PLATE 2

EXPLANATION OF FIGURES

3 A medial longitudinal section through the pyloric node of dog 2, showing the general internal arrangement. A well-defined cortex is present and some of the nodules possess 'germinal centers.' The medullary cords are slender. A dilated medullary sinus (*med.s.*) is shown at one pole. A transitional zone (*tr.zone*), between typical cortex and medulla, resembles the spleen, being marked by irregular areas of erythrocytopoiesis (*a*, *b*, and *c*) and pierced by straight thin-walled blood vessels. The area enclosed in white is shown in figure 4.

4 A portion of the cortex and transitional zone shown under higher magnification. The cortex, according to its staining reactions, may be differentiated in three zones, a peripheral of intermediate intensity (*d*); a middle, deeply stained (*e*), and an inner, lightly stained (*f*). The adjoining transitional zone is characterized by the irregular areas of erythrocytopoietic activity. Note the thin-walled blood vessels (*b.v.*) supplying this region. The area within the square is shown in figure 8.


a
b
c
cortex
tr. zone
periph.s.
med. s.
3

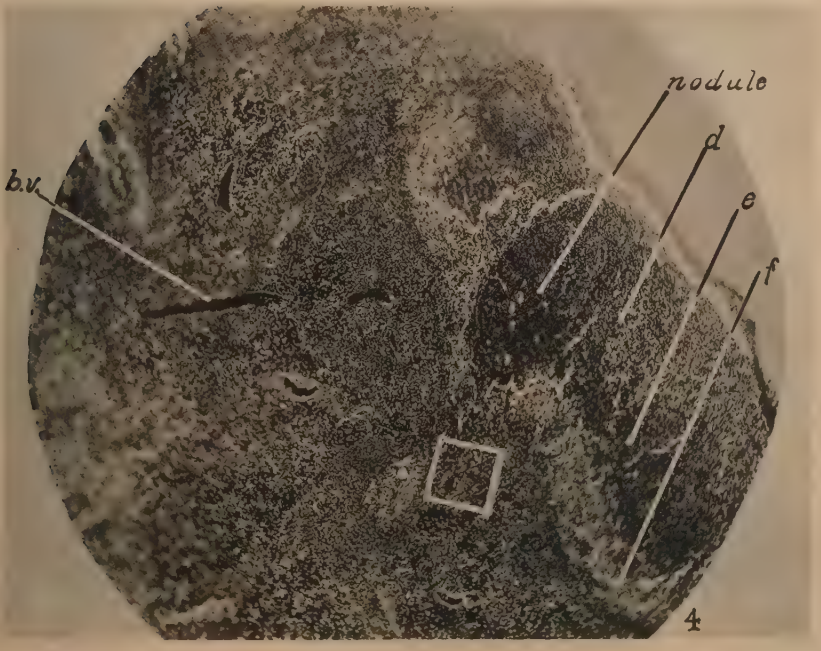
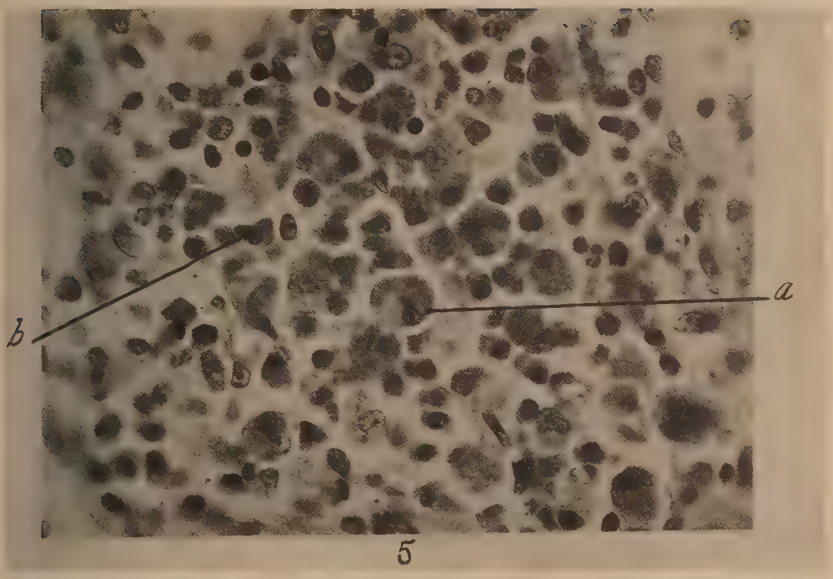

nodule
d
e
f
b.v.
4

PLATE 3

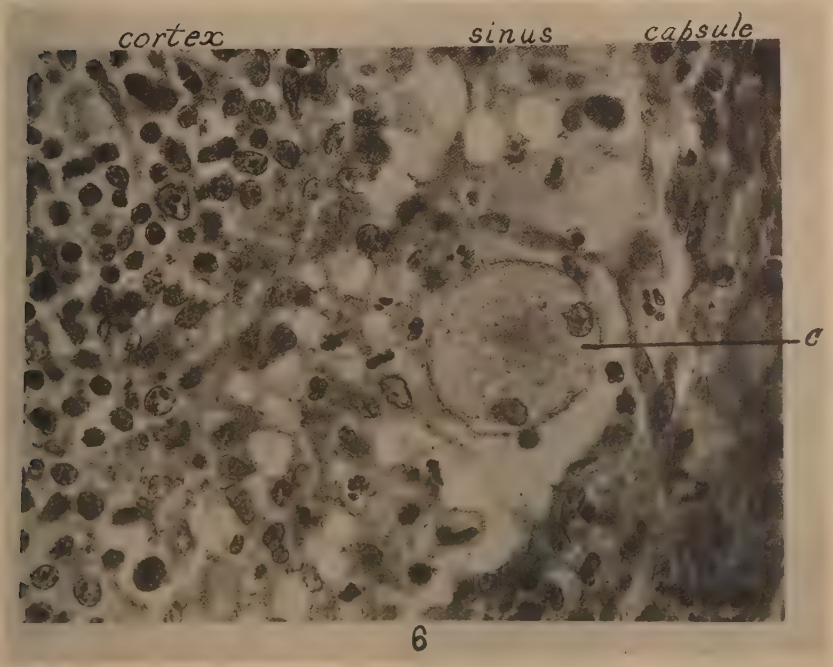
EXPLANATION OF FIGURES

5 An area of the cortex, adjoining the medulla, from the pyloric node of dog 1, showing the differentiation of erythroblasts (*b*) and numerous mononuclear phagocytes (*a*).

6 An area from the peripheral portion of the pyloric node of dog 2, showing the periphery of the cortex, the peripheral sinus, and the capsule. Within the sinus numerous modified reticular cells can be seen. Many possess polymorphic nuclei and in all the protoplasm is pale-staining and highly vacuolated. An extreme condition is presented by the conspicuous binucleate giant cell (*c*). Some of the modified reticular cells in this region exhibit phagocytic activity.



5



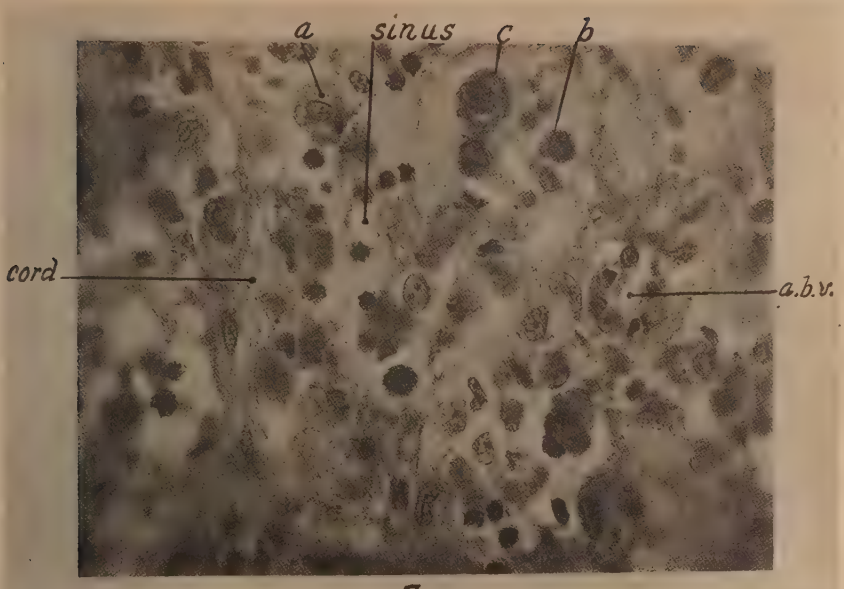
6

PLATE 4

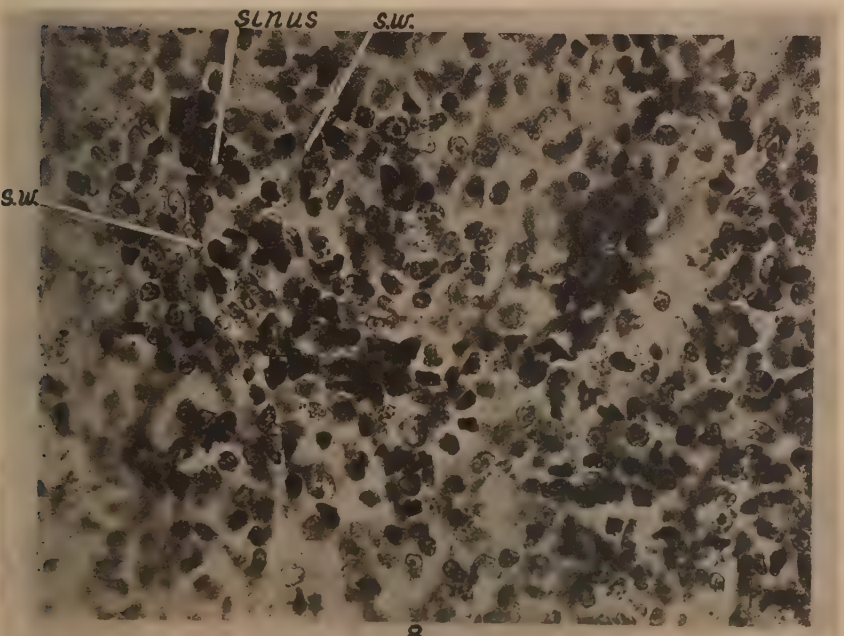
EXPLANATION OF FIGURES

7 A portion of the medullary region of the pyloric node from dog 1. This area is practically devoid of lymphocytes, due to the transformation of these cells into erythrocytes. The sinuses contain scattered lymphocytes, a few normoblasts (*b*), many crenated erythroplastids, and several phagocytes (*a*). The cords contain few free cells and are composed mainly of stroma cells. A degenerating axial blood vessel is shown in the cord on the right (*a.b.v.*).

8 A portion of the transitional zone of the pyloric node from dog 2. The Y-shaped lymph sinus is crowded with cells in all stages of erythrocytopoiesis. The margins of this sinus (*s.w.*) are distinctly shown on the left branch of the Y. The area photographed is indicated by the white square, figure 4.



7



8

PLATE 5

EXPLANATION OF FIGURES

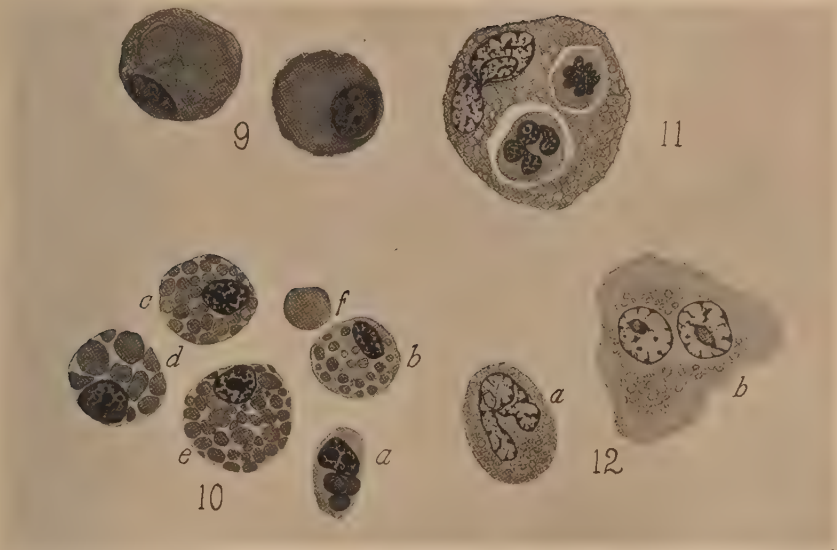
9 Two mononuclear phagocytes similar to those shown in figure 5. These cells are brownish-yellow in stained preparations. The color is presumably due to hemoglobin débris.

10 A group of 'globule' leucocytes selected from the cortex and medullary cords of the pyloric node, dog 1. A red blood cell (*f*) is included to indicate the relative size of these cells. The smallest cell (*a*) contains only three globules, and its cytoplasm is stained with both hematoxylin and eosin, closely resembling a normoblast in this respect. The other four cells, *b*, *c*, *d*, and *e*, contain many more globules, and their cytoplasm is either decidedly eosinophilic or fails to stain.

11 A large, polymorphonuclear, phagocytic giant cell with a highly vacuolated, acidophilic cytoplasm, from the peripheral sinus of the pyloric node from dog 1.

12 Two cells from the medullary sinus of the pyloric node from dog 1. The cell *a* is relatively small, with a polymorphic nucleus and highly vacuolated acidophilic cytoplasm. The cell *b*, of larger size, is binucleate with a perinuclear zone of vacuolation. Compare with cell *c*, figure 7.

13 A cross-section of a slender medullary cord of the mesenteric node from dog 1, showing the axial blood vessels (*a.b.v.*), stroma cells, the reticulum-endothelium bounding the cord and the various stages in the differentiation of erythrocytes. The region from which the drawing was made is indicated by the white circle, figure 1; *a*, lymphocyte; *b*, normoblast; *c*, erythroblast; *d* and *e*, erythroblasts with abnormal nuclei; *f*, stroma cells; *g*, reticulum-endothelial cells.


9
11
c
f
d
b
e
a
10
a
12
b

g
a
g
a
b
c
f
c
e
a.b.v.
c
d
c
c
f
13

THE HEART OF A CHILD AGED TWENTY-TWO MONTHS PRESENTING AN ANOMALOUS VEIN FROM THE PULMONARY AURICLE TO THE RIGHT INTERNAL JUGULAR VEIN, TRANSPOSITION OF THE GREAT VESSELS AND LEFT SUPERIOR VENA CAVA

H. A. HARRIS,[1] S. H. GRAY, AND CAROLINE WHITNEY

Departments of Anatomy, Pathology and Pediatrics, Washington University, St. Louis, Missouri

FIVE FIGURES

A baby boy, aged twenty-two months, was admitted to hospital with bronchopneumonia. He was the only child of healthy parents of twenty-six years of age. The prenatal history was normal, but the accoucheur told the parents that the child had 'heart trouble.' The child was breast-fed for three months and his diet from this time onward was supervised at the hospital clinic. The child sat alone at ten months, cut his first tooth at eleven months, but was unable to walk at twenty-two months, as crying or the slightest effort produced extreme cyanosis; yet he never had convulsions. One week before admission to hospital, the child became feverish and irritable and vomited. On physical examination, the child was seen to be slightly over the average height (62 cm.) and slightly under the average weight (11 kilos). The superficial venules, especially in the conjunctivae, were dilated, and there was moderate clubbing of the fingers and toes. The sternum was convex forward, and Harrison's groove was well marked. The respirations were rapid and shallow, and the physical signs in the chest showed a bronchopneumonia at the base of the left lung. The heart was enlarged

[1] From the Institute of Anatomy, University College, London.

to the left and to the right, the apex beat was 1 cm. outside the nipple line, and the right margin was 1½ cm. outside the right border of the sternum. A harsh systolic murmur was heard at the apex of the heart over an area of greater extent than the usual mitral systolic murmur. There was no thrill. There was an infection of the middle ears, both drums were bulging, and, on paracentesis, yielded thick yellow pus. The temperature was 40.8°C., and the leucocyte count was 28,600. The urine was loaded with albumen and many hyaline casts. Death ensued within twenty-four hours.

POSTMORTEM FINDINGS

The pericardium is normal, and the heart is markedly enlarged to 170 grams, which is twice the normal weight for this age. The enlargement of the right side is the more marked, and the right ventricle occupies almost the whole of the anterior aspect. The aorta arises from the right ventricle and arches to the left to form a normal arch with the three great vessels arising therefrom (fig. 1, *a*). Immediately beyond the origin of the left subclavian artery there is a well-marked coarctation of the aorta, so that its circumference is reduced from 3.8 cm. to 2 cm. The point of maximum coarctation is slightly distal to the point of entry of the ductus arteriosus. The pulmonary trunk arises from the left ventricle and lies posterior and slightly to the left of the aorta. It is 4 cm. in circumference and, after coursing for 2 cm., divides into right and left pulmonary arteries. From a point distant 1 cm. along the left pulmonary artery arises the ductus arteriosus, which runs for a distance of 1.5 cm. to join the aorta. The lumen of the ductus ranges from 3 mm. proximally to 2 mm. distally. The aorta is guarded by three cusps—an anterior and a right and a left posterior—the coronary arteries lying in relation to the two posterior cusps (fig. 1, *d*) instead of to the anterior and left posterior as in the normal (fig. 1, *c*). The aortic cusps are normal, and the line of their attachment measures 3.5 cm. The pulmonary orifice is guarded by three cusps, the total length of which

is 3.2 cm. There are no accessory coronary arteries in relation thereto. The wall of the right ventricle is 6 mm. in thickness, exclusive of the papillary muscle. The right auriculoventricular valve contains three cusps which measure 7 cm. in perimeter; the cusps are thin and delicate. An aberrant

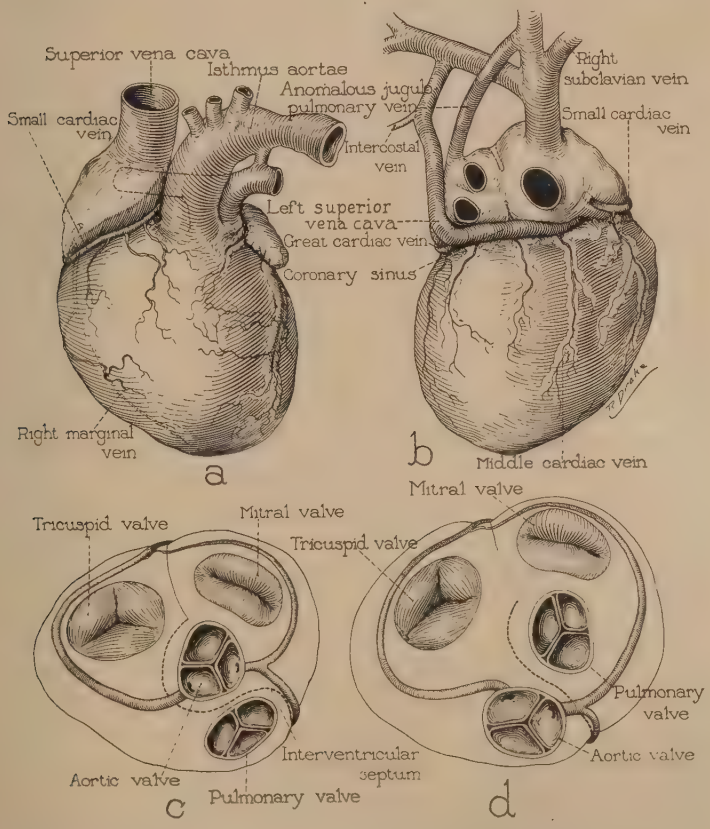


Fig. 1 The heart of a boy of twenty-two months. *a*. Anterior aspect with transposition of the great vessels and coarctation of the aorta. *b*. Posterior aspect with left superior vena cava and the anomalous jugulopulmonary vein. *c*. Normal arrangement of the great vessels and coronary arteries in relation to the interventricular septum. *d*. Abnormal arrangement of the great vessels and coronary arteries in relation to the interventricular septum as a result of incomplete torsion of the pulmono-aortic septum.

chorda tendinea reaches from the anterior wall of the right ventricle to the base of the left posterior aortic cusp, and it gives off a small tendinous band which runs to the anterior surface of the anterior cusp of the tricuspid valve. The left auriculoventricular valve has two cusps which measure 6.5 cm. in perimeter. The trabeculae of the left ventricle and the chordae tendineae are finer than those of the right ventricle. The wall of the left ventricle averages 5 mm. in thickness. The interventricular septum is incomplete, a small foramen of 1 mm. diameter lying near the posterior border 1.5 cm. below the aortic valve.

The venae cavae entering the right auricle are normal; the walls of the latter are from 2 to 3 mm. in thickness, and the cavity of the right auricle is much larger than that of the left, though the walls are of the same thickness. The auricular septum is deficient, for though the limbus fossae ovalis is normal, the foramen ovale is covered by the septum primum in slightly more than half of its extent. In addition to the large opening thus left, there are numerous fenestrations in the septum primum. No opening of the coronary sinus is visible in the right auricle, but the small cardiac vein opens 1 cm. to the right of the inferior vena cava by a valveless orifice. The small left auricle receives two pulmonary veins and displays a rare anomaly which has not been previously observed. This is a vein of 4 to 5 mm. in diameter which runs from the proximal border of the auricle, near the junction of the atrium and the auricular appendage, to the neck. This vein (fig. 1, *b*) is deeply situated and runs deep to the arch of the aorta, crossing the middle line to join the right internal jugular vein just before it joins with the subclavian. This vessel will be called the jugulopulmonary vein. The coronary sinus is normally situated in the auriculoventricular groove at the base of the heart. At the right extremity it ends blindly and fails to communicate with the right auricle; to the left, after receiving the normally disposed veins of the heart, it is continued as a persistent left superior vena cava in the position of the normal oblique vein of Marshall. The

left superior vena cava has a diameter of 4 mm. and receives the left superior intercostal vein. The anomalous jugulopulmonary vein lies deep to the left innominate vein.

Thus the heart presents transposition of the great vessels, coarctation of the aorta, defective auricular and ventricular septa, atypical coronary sinus, persistent left superior vena cava, and an anomalous jugulopulmonary vein of extreme interest.

The other postmortem findings were not of anatomical interest. They included fatty degeneration of the heart and liver, acute bronchopneumonia of the lungs, desquamation of the tubular epithelium of the kidneys, with a granular precipitate in the lumen of the tubules. Three small scars were seen in the kidney, and the spleen showed relatively few malpighian corpuscles with large germinal centers.

ANATOMICAL CONSIDERATIONS

Transposition of the arterial trunks has been analyzed by Théremin(1), Keith(2), and Maude Abbott(3); their results have but served to confirm the brilliant researches of Rokitansky(4). This particular case is an example of complete failure of rotation of the pulmono-aortic septum, so that the aorta is situated anteriorly and arises from the right ventricle, whereas the pulmonary trunk is posterior and arises from the left ventricle. The site of origin of the coronary arteries (fig. 1, *d*) confirms this. The need for further knowledge of the spina vestibuli of His in relation to the lower end of the pulmono-aortic septum has been mentioned in a recent article by Harris and Whitney(5). If, as Keith suggests, the upper border of the interventricular septum represents the least disturbed part of the lumen of the embryonic cardiac tube, it is also true that the primitive venous valves represent the most disturbed part of the cardiac tube. The primitive venous valves not only give rise to the valves of Eustachius and Thebesius, but also, at their lower point of fusion, to the spina vestibuli. The spina vestibuli in turn enters into the formation of the auricular and ventricular septa and into the lower portion of the pulmono-aortic system.

The results of transposition in this case are seen in figure 2. The systemic and pulmonary circulations are closed circuits. Systemic blood never reaches the pulmonary side of the heart, and pulmonary blood never reaches the aortic side of the heart but for the presence of several shunts. In the foetal circulation there are two shunts: the patent foramen ovale within the heart and the patent ductus arteriosus outside the heart. The nature of the mechanism at these shunts has remained equivocal since the time of Hippocrates and the time of Harvey. The work of Pohlman(6) has shown the limitations of the older experimental methods, but the recent experiments of Swenson(7) have indicated a new method of approach inasmuch as he has succeeded in keeping the embryo of the rat alive for several hours while lying outside the mother with the circulation intact. In addition to the two normal shunts of foetal life, this two-year-old boy has maintained a third foetal shunt in the form of a foramen in the ventricular septum, and has acquired a fourth shunt in the form of the most atypical jugulopulmonary vein. The sum total of the admixture of arterial and venous blood by means of these four shunts must have been adequate to maintain life in spite of the apparently closed circulations consequent upon the transposition of the great vessels.

The anomalous jugulopulmonary vein appears to have no counterpart in comparative anatomy, and no case has been found in which the systemic veins are directly connected to the pulmonary ventricle. Numerous cases of communication between the pulmonary veins and the systemic veins have been described, especially by Gruber(8), Thane(9), Ingalls(10), Johnston(11), and Schroeder(12). Since the trachea and lungs are developed from the primitive foregut, it is to be expected that the veins of the lungs should show the same type of irregularities in relation to the systemic veins as do those of the foregut. Brown(13), in his study of the early pulmonary veins of the cat, shows that embryology gives the explanation of anomalous pulmonary veins which comparative anatomy fails to give. Brown appreciated to the full the

valuable dictum of Zuckerkandl(14) to the effect that the pulmonary veins are remnants of an originally vast indifferent plexus of capillaries in relation to the foregut from which lines of drainage have been developed as best suited

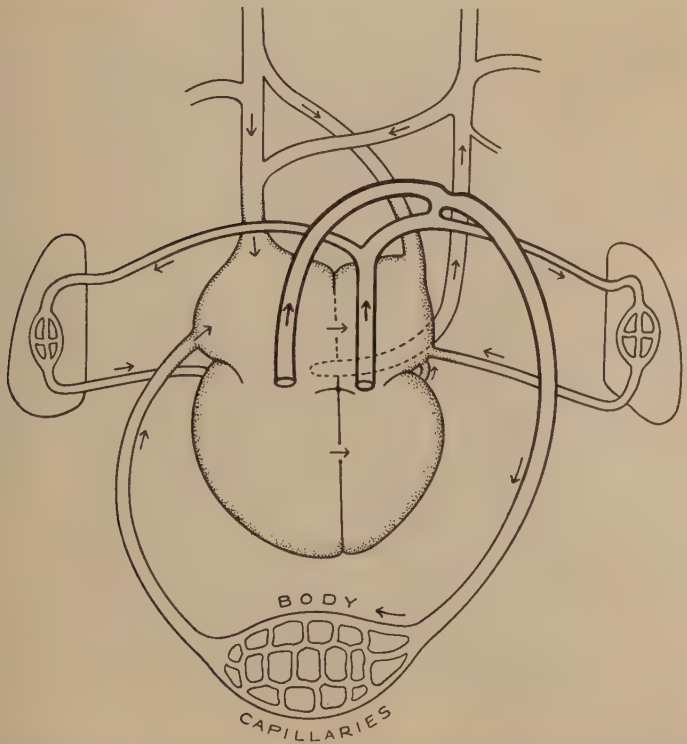


Fig. 2 Schema of the circulation of the boy's heart, to show the four shunts between the closed systemic and pulmonary circulations viâ the auricular septum, the ventricular septum, the ductus arteriosus, and the anomalous pulmonojugular vein.

to the functions of the part. The reconstruction of the 4.5-mm. cat shown by Brown presents a sinus venosus which receives two veins from the gut plexus—the cephalic and caudal 'taps,' as he calls them (fig. 3). The gut plexus in turn anastomoses freely with the anterior and posterior cardinal veins. The

cephalic 'tap' becomes the definitive common pulmonary vein and the caudal 'tap' becomes the hepatic portion of the future postcava. Thus the common pulmonary vein is connected at an early age with the veins of the foregut and with the cardinal system by several anastomoses, of which two have been strengthened in the figure to indicate their possible persistence. The persistence of any one of these channels accounts for all the irregularities described in the way of abnormal communications between the pulmonary veins and the cardinal system, both as regards the superior vena cava and as regards the inferior vena cava, as in the case described by Park(15), where the lower lobe of the left lung drained into the inferior vena cava. The former are cases of persistence of the cephalic 'tap' and the latter of the caudal 'tap.' In the process of growth of the left auricle and in the process of differentiation of the common pulmonary vein to form the paired pulmonary veins, such anastomotic taps may come to be incorporated in the left auricle. It is suggested that the atypical jugulopulmonary vein of the case under consideration is a persistence of the primitive anastomotic channel which at the cephalic end has retained its attachment to the anterior cardinal vein—now the internal jugular—and at its caudal end has become incorporated into the wall of the left auricle. It is difficult to postulate the direction of blood flow in such a channel, but the small size of the left auricle suggests that blood flowed from the left auricle through this vein to the internal jugular vein, and so to the right heart and aorta. On the other hand, the pressure in the systemic venous system is greater than that in the pulmonary venous system and in the left (pulmonary) auricle, so that blood must flow from the right internal jugular vein to the left auricle.

The persistent left superior vena cava in this case differs from Marshall's (16) classical type, inasmuch as the direction of the blood flow is reversed, by reason of the complete obliteration of the opening of the coronary sinus in the right auricle. Cases of this reversed blood flow in the left superior

vena cava have been described by Le Cat(17), Grüber(18), Anton Siding(19), Nabarro(20), Hutton(21), and Beyerlin (22). The condition is usually attributed to an increased degree of fusion of the lowermost thirds of the primitive

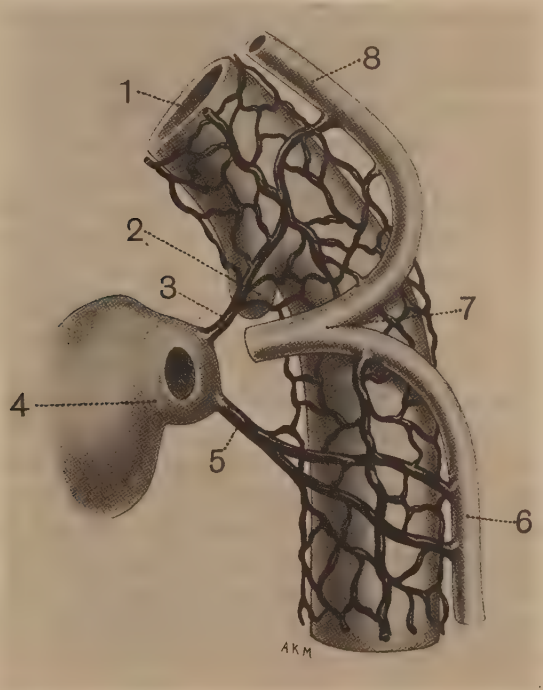


Fig. 3 Reconstruction of a 4.5-mm. cat embryo (after A. J. Brown). *1*, foregut; *2*, lung bud; *3*, cephalic 'tap'; *4*, sinus venosus; *5*, caudal 'tap'; *6*, postcardinal vein; *7*, duct of Cuvier; *8*, anterior cardinal vein. The cephalic and caudal 'taps' show how the future pulmonary veins and the future inferior vena cava anastomose viâ the gut plexus with the anterior and posterior cardinal veins.

venous valves, so that the opening of the coronary sinus fails to persist. Normally, the small cardiac vein opens into the right end of the coronary sinus, but in this case it opens directly into the right auricle by a small opening which is not guarded by any valve. This condition was described

by Watson(23) as the normal arrangement in the elephant. It is suggested that the anomalies of this area depend on the extent to which the sinus venosus is taken up into the wall of the right auricle. A persistent left superior vena cava tends to be associated with a small left auricle, as is seen in birds and reptiles. Those animals in which there is a persistence of the left superior vena cava or of a left vena azygos which joins the coronary sinus are always devoid of a thebesian valve at the orifice of the coronary sinus. This is seen in all except the higher mammalia and the primates in which the presence of the thebesian valve is associated with suppression of the left superior vena cava. It is difficult to suggest what irregular differentiation of growth has taken place in the enlarged right auricle of this infant, but the separation of the coronary sinus and the small cardiac vein indicate that either there has been a peculiarly localized overgrowth of the heart wall in this area or the sinus venosus has been taken up by the right auricle to an abnormal extent.

It is remarkable that persistence of the left superior vena cava, which is by no means uncommon in man, should be so rare in the domesticated animals of the carnivore group. Considering the very large number of cats and dogs used for experimental purposes, and that a large number of these experiments have involved operations in the neighborhood of the great vessels of the heart, it is significant that only two cases of the condition should have been reported. Both occurred in dogs—one reported by Bradley(24) and the other by Harris(25). One is tempted to regard this as yet another example of that persistence of foetal characters which distinguishes man from the other primates and the Carnivora.

The mild degree of coarctation of the aorta in this case is of Bonnet's(26) 'infantile type,' because of the patency of the ductus arteriosus in association with anomalies of the heart, cyanosis, and clubbing of the fingers. The degree of coarctation of the aorta is not very marked and there are no signs of a collateral arterial circulation. Yet a recent rapid progression of this degree of coarctation may have been an

effective agent in the overloading of a circulation which was already working under manifest disadvantages. Of the four shunts which were instrumental in maintaining life in this case, the only one which is likely to have undergone any marked modification after the first month of life is the shunt through the ductus. Skoda(27) was the first to suggest that the ductus, richly supplied with muscular tissue, was endowed with an endogenous progeria, whereby it rapidly became subject to senile changes early in postnatal life. Further, Skoda suggested that coarctation of the aorta was due to the rapid aging and fibrosis of the muscle in that portion of the ductus (VIth arch) which had become incorporated in the wall of the aorta in the region of the junction of the ductus with the transverse portion of the aorta (IVth arch). Thus it appears possible that in this case the progressive degree of coarctation may have been the determining factor in the breakdown of a circulation which was already overburdened by reason of the transposition of the great vessels.

THE REVERSAL OF BLOOD FLOW IN CONGENITAL HEART DISEASE

There is no definite experimental evidence for the accepted description of the course of the foetal circulation. The classical views of Haller have been repeatedly challenged, but Pohlman(6) and Ziegenspeck(28) have not succeeded in producing a change of view with regard to this mechanism. The volume of the various chambers of the heart, the volume of the blood circulating through the right and left sides, and the changes of pressure in the pulmonary circulation are still but imperfectly known. None of the purely mechanical factors which have been employed in the pathology of congenital heart disease have met the position. The work of Holman(29) and of Reinhoff(30) on patent ductus arteriosus as an arteriovenous shunt has elucidated certain points, but there is still a startling discrepancy between the clinical forecast of the lesion and the actual postmortem findings. Lewis and Drury(31) have shown experimentally that the escape of blood from artery to vein, in the case of large trunks, does

not necessarily or even customarily raise the general venous pressure. This is so because a free escape leads to a lowering of the general arterial pressure, and less blood is consequently forced through the capillaries. Lewis and Drury state that in the case of a free arteriovenous shunt the enlargement of the heart is due to deficient nutrition of the heart muscle consequent upon the fall of mean arterial pressure.

As to the ductus arteriosus itself, omitting the effect of the other possible shunts, the purely mechanical effects are represented in figure 4. The immediate antenatal and postnatal conditions are shown in *a* and *b*; in *c* and *d* are shown the two possible postnatal conditions in congenital heart disease. In one case the blood flows from the right ventricle viâ the ductus to the aorta; in the other, blood flows from the arch of the aorta viâ the ductus to the pulmonary circulation. In this case the direction of blood flow is the reverse of that which holds during foetal life. In the case of a patent ductus with the blood flowing in the foetal direction, the volume of blood traversing the right heart is increased. Such a heart should display an enlargement of the right side and of the tricuspid ring, a dilatation of the pulmonary trunk and of the aorta immediately distal to the ductus. The descending aorta contains imperfectly oxygenated blood (AV), but the ascending aorta contains richly oxygenated blood (A), so that the nutrition of the heart viâ the coronary arteries is normal. On the other hand, with reversal of the direction of the blood flow through the ductus, the flow of blood through the left heart is greater than that through the right heart. Such a heart should show enlargement of the left side and a dilatation of the aorta proximal to the ductus. The aorta contains richly oxygenated blood and cyanosis should be absent, as distinct from the former case where cyanosis is indicated. A survey of the cases enumerated by Maude Abbott(3) and in particular of cases with a careful report of the two sides of the heart, such as that of Langdon Brown and Durno(32) show that the mechanical factors fail to explain the physical signs or the pathological findings.

If we apply this purely mechanical conception to the case of a patent ductus in association with transposition of the

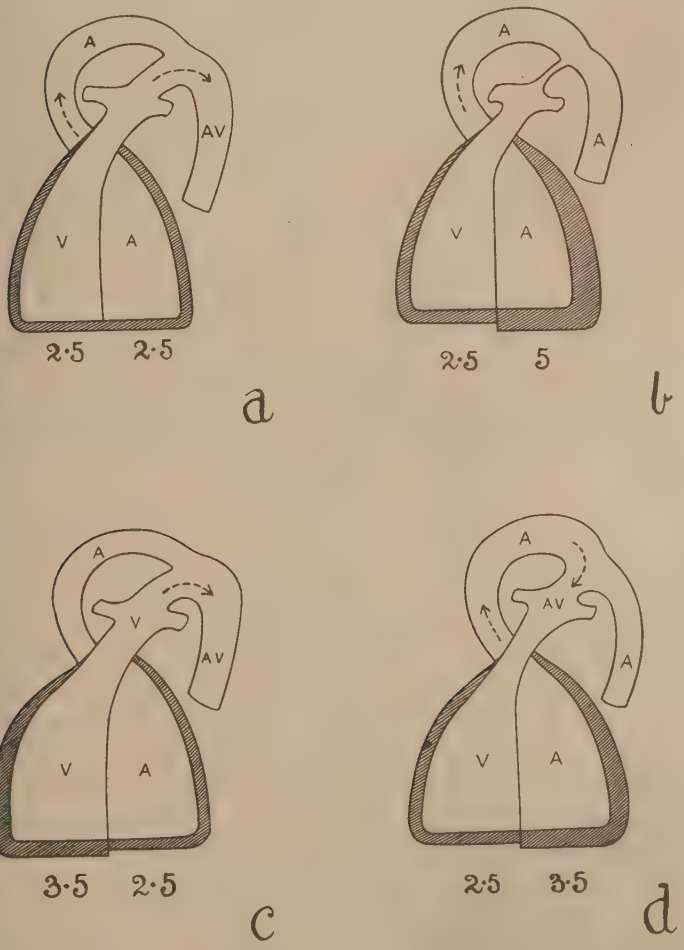


Fig. 4 The direction of the flow of blood through the patent ductus arteriosus in: *a*. The normal heart just before birth. *b*. The normal heart at one month after birth. *c*. Congenital heart with normal direction of blood flow in the ductus. *d*. Congenital heart with reversed direction of blood flow in the ductus. The thickness of the ventricular walls is shown in millimeters.

great vessels, then, according to the direction of blood flow in the ductus, the condition will be as shown in figure 5, *a* and *b*. In the former condition the left (pulmonic) side of the heart is enlarged and there is a dilatation of the aorta distal to the ductus; in the latter condition there is a dilatation of the aorta proximal to the ductus with enlargement of the right (systemic) side of the heart. In the former case the descending aorta contains mixed blood (*AV*), whereas in the latter it contains purely venous blood. The former is associated with an increased blood flow through the left heart and the latter with an increased flow through the right heart. The one shows cyanosis and an enlarged left auricle; the other, extreme cyanosis and a small left auricle.

The actual heart under consideration (fig. 5, *c*) is a clear demonstration of the difficulty of interpreting the condition in terms of mechanical factors. Since the pulmonary trunk is dilated to a circumference of 4 cm. and is larger than the ascending portion of the arch of the aorta, and since the left (pulmonary) ventricle is hypertrophied to 5 mm., which is about twice the normal for this age, it would appear that the flow of blood through the left heart is greater than that through the right heart. This indicates that the flow of blood through the ductus in this case is from the pulmonary viâ the ductus to the aorta. On the other hand, since the aorta has a circumference of 3.8 cm. immediately proximal to the ductus and since the right (systemic) ventricle is hypertrophied to 6 mms., it would appear that the flow of blood through the ductus is from the aorta to the pulmonary. A fairly rapid increase in the process of coarctation of the aorta in the few weeks before death may have led to a recent dilatation and hypertrophy of the right side, so accounting for the large right auricle and for the slight increase in the size and thickness of the right ventricle. In all the conditions shown in figure 5 the heart is under a great disadvantage as the coronary arteries arise from a systemic ventricle which contains venous blood as a result of the transposition.

If we consider the circulation shown in figure 4, *d*, it can be seen that some of the arterial blood in the arch of the aorta is short-circuited through the ductus to the pulmonary circulation, so that it is returned to the lungs for oxygenation without having had an opportunity of surrendering its oxygen

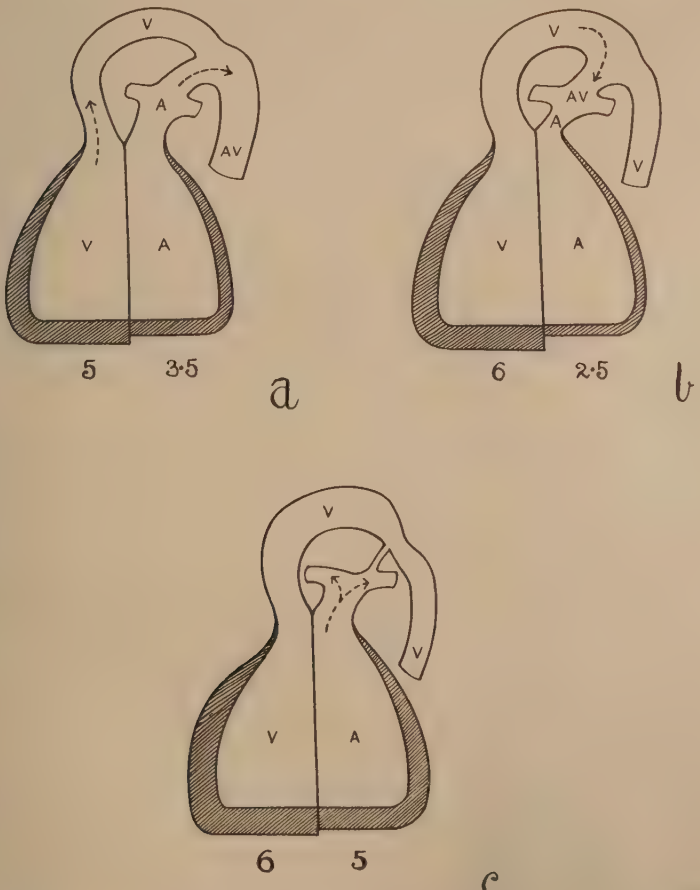


Fig. 5 Congenital heart with transposition of the great vessels. *a*. Normal direction of flow through the patent ductus. *b*. Reversed direction of flow through the patent ductus. *c*. The condition found in the boy's heart. The thickness of the ventricular walls is shown in millimeters.

to the tissues at the periphery. Such a waste in the transportation system can be remedied by two distinct compensatory mechanisms. In the first place, the number of cells engaged in the transport of oxygen can be increased from five million to seven or ten million per cubic centimeter, according to the amount of the blood which is short-circuited; this is determined by the ratio of the lumen of the ductus to that of the aorta. In the second place, the speed of transportation of the red cells can be increased by an increase of the blood volume. Thus the compensatory mechanism consists of an erythraemia and an increase of the blood volume, so that there are more corpuscles carrying oxygen and their rate of transport is increased. The erythraemia of these cases has been repeatedly discussed by Leslie(33), Parkes Weber(34), Vaquez(35), and Osler(36) among others. On the other hand, the information regarding blood volume in cases of congenital heart disease is almost negligible, and the need for such is pressing. This short-circuiting of blood in the reverse direction of flow through the ductus may be a normal phenomenon in the first few weeks of postnatal life, and its existence over a period of several weeks in the normal process of closure of the ductus may provide one of the main stimuli to that proliferation of the bone marrow which characterizes the first few weeks of life. The activity of the newborn colt, lamb, or leveret is strikingly different from that of the newborn babe, rabbit, or rat. The actual changes in the circulation of these forms at birth have never been comparatively studied.

In the case of patent ductus arteriosus with transposition of the great vessels (fig. 5, *a*), the persistence of the normal direction of blood flow delivers arterial blood to the periphery, but also delivers arterial blood to the lungs without its reaching the periphery in the meantime. Such a circulation appears to be quite incompatible with life apart from the existence of additional shunts. As may be seen in figure 2, such a case has two closed circulations for the pulmonary and systemic systems. In the case of reversed blood flow

through the ductus (fig. 5, *b*), arterial blood from the left ventricle is sent to the lungs directly as in the previous case, but in addition to the two closed circuits there is now a third circuit whereby venous blood travels from the right ventricle to the arch of the aorta and then viâ the ductus to the lung. This is the very opposite in its effect to the case of reversed blood flow in the common type of patent ductus. In the latter arterial blood is short-circuited from the aorta viâ the ductus to the lungs, and so calls for an increase of the red cells and an increase of the blood volume. In the former case (fig. 5, *b*), the venous blood in the aorta is sent viâ the ductus direct to the lungs for reoxygenation. In other words, the reversal of blood flow through the ductus is a physiological advantage in the heart of this child.

As soon as the other shunts by way of the patent foramen ovale, ventricular foramen, and the anomalous jugulopulmonary vein are brought into consideration, the whole of the mechanism discussed for figures 4 and 5 becomes very weak. There is probably a large leak of venous blood from the right internal jugular vein viâ the jugulopulmonary vein to the left (pulmonary) auricle, and so to the left ventricle and the lungs for oxygenation. Again the flow of venous blood from the aorta to the lungs viâ the ductus is advantageous to the heart concerned, but the actual conditions of the heart and great vessels do not clearly indicate that the flow was from the aorta through the ductus to the pulmonary. In fact, there seems to be great difficulty in postulating anything of the circulation in a case of congenital heart lesion. The reported cases of congenital heart have been analyzed from the point of view of ascertaining if there were any parallelism between the postmortem findings with a view to forecasting the nature of the anomaly from the physical signs. As yet there is little hope of success. The one thing that emerges from the study of the heart of this child is that there is apparently no limit to the means which the organism can employ to overcome an existing defect. The development of a vein from the left auricle to the internal jugular in this case

was a masterly stroke of embryological opportunism and served to keep this child alive for twenty-two months. On the clinical side there is an urgent need for blood-volume determinations in all cases of congenital heart disease.

We are indebted to Mr. Drake, of the Mayo Clinic, for figure 1 and to Mr. Maxwell, of University College, London, for the others.

BIBLIOGRAPHY

1 Thérémin 1895 Études sur les affections congénitales du coeur. Paris.
2 Keith 1909 Lancet, vol. 2, pp. 359, 433, 519.
3 Abbott, Maude E. Congenital heart disease. Osler and McCrae's System of Medicine, vol. 4, p. 379.
4 Rokitansky 1875 Defekte der Scheidewände des Herzens. Vienna.
5 Harris and Whitney 1927 Anat. Rec., vol. 34, pp. 221–232.
6 Pohlman, A. G. 1907 Johns Hop. Hosp. Bull., vol. 18, pp. 409–412.
7 Swenson, E. A. 1925 Anat. Rec., vol. 30, pp. 147–151.
8 Gruber 1876 Virchow's Arch., Bd. 68, S. 284–287.
9 Thane 1906 Jour. Anat. and Phys., vol. 40, p. 11.
10 Ingalls, N. W. 1907 Anat. Rec., vol. 1, p. 14.
11 Johnston, T. B. 1915 Jour. Anat. and Phys., vol. 49, pp. 182–186.
12 Schroeder 1911 Arch. f. klin. Med., Bd. 205, S. 122.
13 Brown, A. J. 1913 Anat. Rec., vol. 7, pp. 299–330.
14 Zuckerkandl 1881 Sitzungberichte der kais. Akad. der Wissensch., Bd. 84, S. 1.
15 Park, E. A. 1912 Proc. New York Path. Soc., new series, vol. 12, p. 88.
16 Marshall 1850 Phil. Trans. Roy. Soc., part 1, pp. 133–170.
17 Le Cat 1738 Histoire de l'acad. roy. des Sciences. Paris.
18 Gruber 1885 Virchow's Arch., Bd. 99, S. 492.
19 Siding, Anton 1896 Anat. Anz., Bd. 12, S. 274.
20 Nabarro 1902 Jour. Anat. and Phys., vol 37, p. 387.
21 Hutton 1915 Jour. Anat. and Phys., vol. 49, p. 407.
22 Beyerlin 1914 Frank. Zeit. f. Path., Bd. 15, S. 237.
23 Watson 1872 Jour. Anat. and Phys., vol. 6, p. 82.
24 Bradley, O. C. 1902 Anat. Anz., Bd. 21, S. 142.
25 Harris, H. A. 1921–1925 Proc. Anat. Soc. Gt. Brit. and Ireland, p. 59.
26 Bonnet 1903 Révue de Médecine.
27 Skoda Quoted by Abbott(3) in Osler and McCrae's System of medicine, vol. 4, p. 422.
28 Ziegenspeck 1910 Gyn. Rundschau, Bd. 4, S. 75–109.
29 Holman, E. 1925 Johns Hop. Hosp. Bull., vol. 36, pp. 61–80.
30 Reinhoff, W. F. 1924 Johns Hop. Hosp. Bull., vol. 35, pp. 271–284.
31 Lewis and Drury 1923 Heart, vol. 10, p. 364.
32 Durno and Brown 1908 Lancet, vol. 1, p. 1692.

33 LESLIE, MURRAY 1908 Proc. Roy. Soc. Med. Clin. Sect., vol. 1, p. 34.
34 WEBER, PARKES 1921 Polycythaemia, erythrocytosis and erythraemia. London.
35 VAQUEZ 1904 Compt. Rend. de la Soc. de Biol., T. 57, p. 135.
36 OSLER 1908 Lancet, vol. 1, p. 143.

JAN 7 1923
LIBRARY

THE EARLY POSTNATAL DEVELOPMENT OF THE PATELLA OF THE DOG (CANIS FAMILIARIS)

EBEN J. CAREY AND WALTER ZEIT

Department of Anatomy, Marquette University School of Medicine, Milwaukee, Wisconsin

ELEVEN FIGURES

INTRODUCTION

The object of this paper is to present the following changes that occur in the patella during the formation of the endochondral bone:

1. That ossification begins in the center of the cartilaginous patella comparable to that found in the development of the epiphyses of long bones.
2. That the osteoblasts have a double origin: some are derived from the subendothelial mesenchymal cells, others by the direct transformation of young embryonic cellular cartilage.
3. That there is a double arrangement of the trabeculae of bone in the patella, which apparently corresponds to stress lines of pressure and tension.

THE PERIOD OF THE CHONDROGENOUS PATELLA

The patella of a six-weeks-old fox-terrier puppy is composed of vascularized hyaline cartilage (fig. 1). This embryonic hyaline cartilage is a histogenetic product of the undifferentiated scleroblastema. The ectoplasm of the cells forming the scleroblastema becomes thickened and the nuclei surrounded by endoplasm eventually lie in spaces formed by the ectoplasm. Underlying the perichondrium there is an incipient condensation stage known as precartilage, proto-

1

2

3

4

cartilage, or parenchymatous cartilage of Schaefer. The matrix of this precartilage takes on the characteristics of embryonic hyaline cartilage. The ground-substance forms an attenuated wall for each cell. The embryonic cellular cartilage is composed of vesicular spindle-shaped and irregular rounded cells between which is formed the chondromucoid ground-substance. Toward the center of the patella this ground-substance or matrix increases in amount.

The dimensions of the fixed patella of a six-weeks-old fox-terrier puppy are as follows: dorsoventrad, 11.8 mm., and through the largest diameter cephalocaudad, 5.2 mm. (fig. 1). On the convex ventrocephalic aspect of the patella there is a fibrous perichondrium. Underlying this is the osteogenetic vascularized cellular zone which indefinitely blends with the embryonic precartilage. The cartilage cells near the perichondrium are lineated along oblique lines corresponding to strands of white collagenous fibers which extend from the perichondrium into the substance of the cartilaginous patella.

The blood vessels in the cartilaginous region underlying the perichondrium are directed obliquely, corresponding to the long axes of the strands of collagenous fibers from the perichondrium. Toward the middle of the cartilaginous patella a network of blood-vascular capillaries is found. Each blood vessel is surrounded by irregular groups of cartilaginous cells, the matrix of which is more increased in amount than in the region immediately underlying the perichondrium.

On the dorsocaudal, concave, and articular aspect of the patella the cartilaginous nuclei are elongated, flattened, and more closely compact than the nuclei found in the middle of

Figs. 1, 2, 3 and 4 Photomicrographs of patellae of six-, six-and-one-half-, seven-and-one-half-, and nine-and-one-half-weeks-old fox-terrier puppies. The plane of section is sagittal, or cephalocaudad, in the dog; 7.5 times natural size of the fixed specimen. The most salient feature to be noted is an actual diminution in size of the patellae from the six- to the nine-and-one-half-weeks-old stages. This condensation or decrease in volume is coincident in time with the progressive ossification of the patellae. The perichondrial or periosteal aspect of the patella is to the left and the cartilaginous articular aspect to the right. These photomicrographs are untouched and have all been taken with the same magnification. (Iron hematoxylin and eosin.)

the cartilaginous patella (fig. 5). The long axes of the flattened or spindle-shaped nuclei are directed dorsoventrad corresponding to the long axis of the articular surface of the

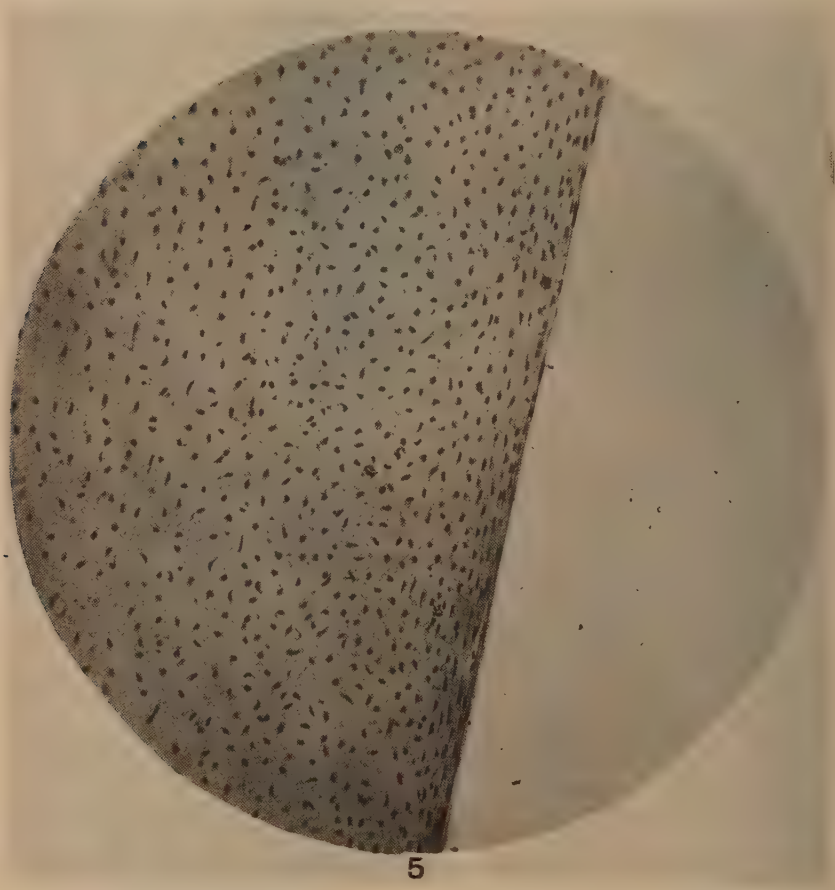


Fig. 5 The articular aspect of the patella of a six-and-one-half-weeks-old fox-terrier puppy. From the articular to the deep aspects of the patella the nuclei become more ovoid, spindle-shaped, and spherical. In many places vacuoles are found in close association with normal nuclei. The matrix increases in amount from the articular to the deep aspects of the patella. (Iron hematoxylin and eosin.) × 75.

patella. This flattened articular zone is composed of from four to eight layers of cells. Toward the center of the patella and underlying the flattened zone of nuclei, the nuclear forms are more stelate and ovoid, depending upon the plane of section.

The intercellular cartilaginous matrix progressively increases in amount from the articular aspect toward the middle of the patella, in the same way that it increases in amount from the perichondrial side toward the middle. Toward the middle or center of the patella the cartilaginous cells are arranged in irregular-shaped lacunae containing one to several nucleated cells. Immediately surrounding the endothelium of blood vessels the intercellular cartilaginous matrix has an orange-pink staining reaction that irregularly radiates from the blood vessel as a center (fig. 6). Farther away from the blood vessel the intercellular matrix gradually changes from orange-pink to a cerulean blue. The canalized blood vessels which extend into the cartilage are preceded in many locations, studied in serial sections, by solid cords of vascular mesenchyme.

In many places there are found vacuoles with diameters ranging from 1 μ to 15 μ. These vacuoles directly border the nucleated cells (fig. 6). The cells bordering the larger vacuoles have no evidence of degeneration, but are, however, concentrically flattened around the periphery of the vacuole. In certain places these vacuoles are seen clearly to originate within the endoplasm of the cartilaginous cells.

THE PERIOD OF INCEPTION OF NECROBIOSIS OF THE CARTILAGE CELLS

The density of the perichondrium becomes more marked as development progresses. Immediately underlying the summit of the dense perichondrium on the convex aspect and toward the center of the patella there are found numerous enlarged vascular spaces (fig. 2). This is comparable to the development found in the pig's femur by Carey ('22). These changes are found in a six-and-one-half-weeks-old fox-terrier

puppy, the patella of which measures 10.6 mm. dorsoventrad and 4 mm. through the largest diameter cephalocaudad. The patella at this stage is slightly smaller than the preceding

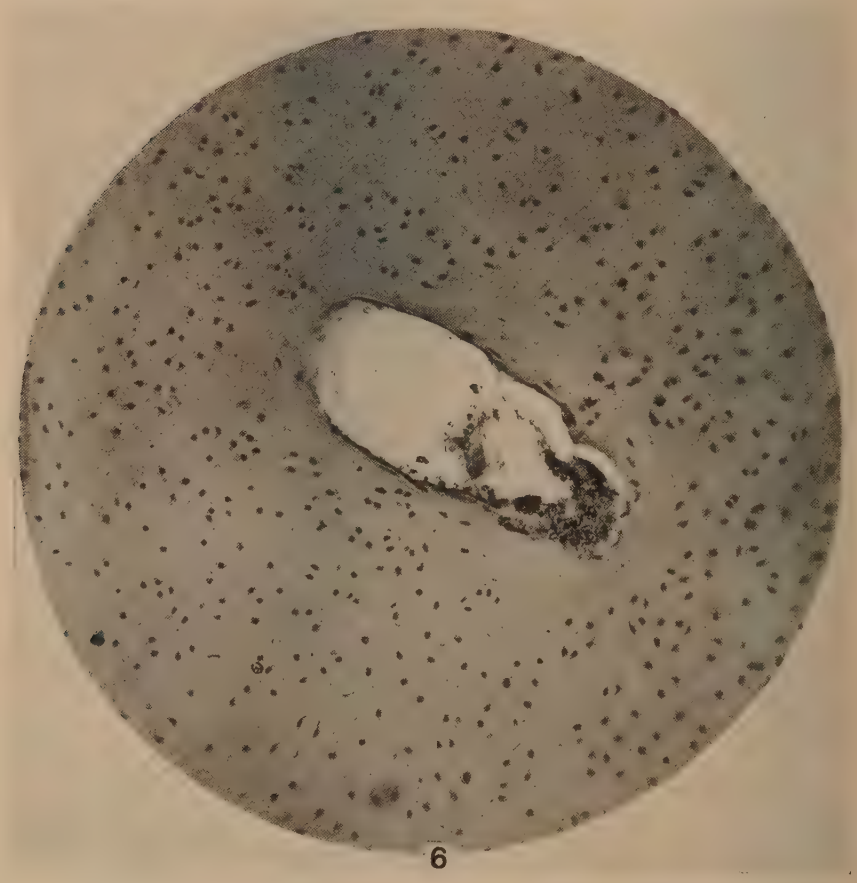
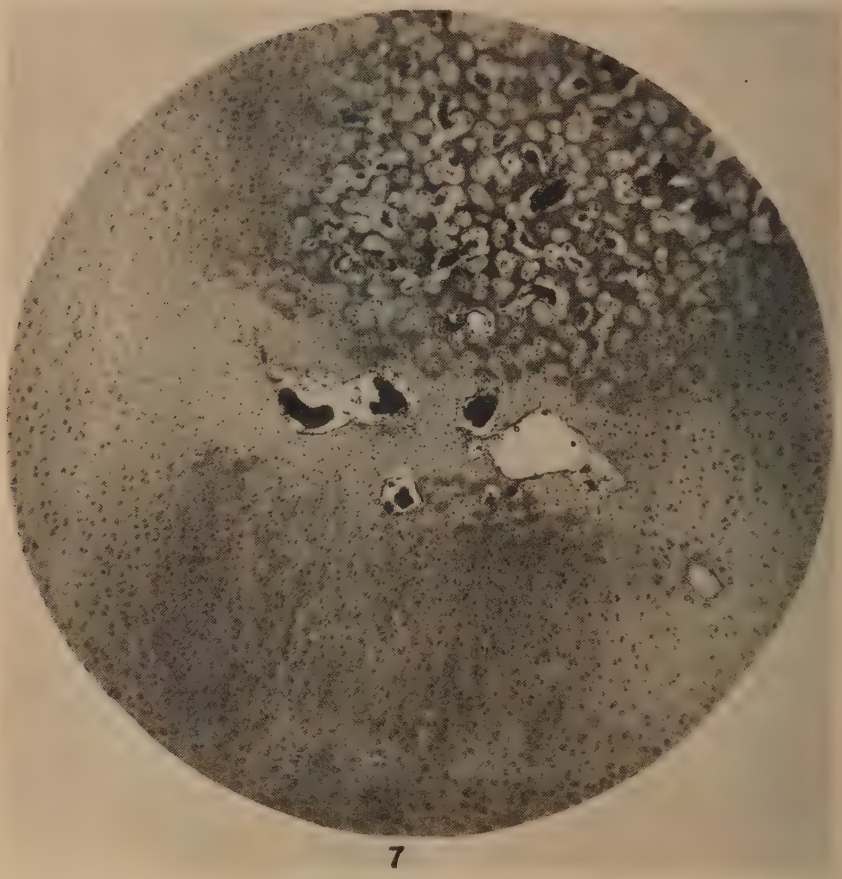


Fig. 6 The cytoplasm of the subendothelial osteoblasts gradually blends with the broad rim of cartilage matrix surrounding the blood vessels. This matrix is orange-pink, comparable to osteoid tissue. The cartilage cells are arranged in irregular groups. In many places the cytoplasm of the cells has vacuoles in close relationship with nuclei, ranging from 1 μ to 15 μ in diameter. The nuclei bordering these vacuoles have no evidence of degeneration. (Iron hematoxylin and eosin.) $\times$ 75.

one, in spite of the fact that it is older both chronologically and anatomically. This period represents the beginning of the series of transformations leading to the condensation of the patella or ossification.

At the rim of the primary center of patellar ossification there are seen large vascular places surrounded by an irregular network of cartilage matrix (fig. 7). The intercellular matrix is not impregnated with calcium salts at this stage, evidenced by the lack of deep blue staining reaction; therefore, no calcification of the matrix. This vacuolization of cartilage prior to calcification is like that observed in the chick bones by Retterer ('00). The cross-sections of some of the blood vessels have many red and white blood cells in their lumina. The walls between the vacuoles are fairly broad in some places, thinner in others, and in certain places totally absent. The larger blood vessels at the caudal border of the center of ossification are surrounded by a broad matrix rim of orange-pink, comparable to that found in osteoid tissue. These osteoid cells gradually and indefinitely blend with the more outlying cells of embryonic cellular cartilage. These osteoid cells also indefinitely blend with the proliferating cells underlying the endothelium of the blood vessels (fig. 7).

This gradual transition of osteoid tissue into cartilage on the one hand and with the underlying cells of the endothelium on the other is quite evident in higher powers of magnification (fig. 8). In this photomicrograph the orange-pink osteoid tissue appears light surrounding the vascular spaces. Many of the cells in this osteoid zone have the typical morphology and structural arrangement of cartilage cells and are morphologically indistinguishable from the typical cellular hyaline cartilage that has a cerulean blue matrix. The direct transformation of these embryonic cartilage cells into bone is quite similar to the formation of bone from the proliferating group of cells underlying the endothelium of the blood vessels. Only in certain places more peripherad to the primitive osteoid tissue and the transforming cartilage into bone is there evidence of vacuolar degeneration of the car-



7

Fig. 7 Photomicrograph of the patella of a six-and-one-half-weeks-old fox-terrier puppy. This enlarged view is taken from the caudal end of the beginning of the center of ossification shown in figure 2. Immediately surrounding the blood vessels, the cytoplasm and matrix of the cartilage have an orange-pink appearance comparable to osteoid tissue; more peripherad to the blood vessels, the matrix has a light blue appearance, which is dark on the photomicrograph. The cells composing the osteoid zone, immediately surrounding the blood vessels, gradually and indefinitely blend with the outlying embryonic cellular cartilage on one side and with the proliferating subendothelial cells of the blood vessels on the other. (Iron hematoxylin and eosin.) × 50.

tilage. Evidently, then, close proximity of young embryonic cellular cartilage to vascular spaces is a determining factor in the transformation of these cartilage cells into osteoblasts (fig. 8). A festooned appearance results from the progressive branching of the blood vessels and extension into the

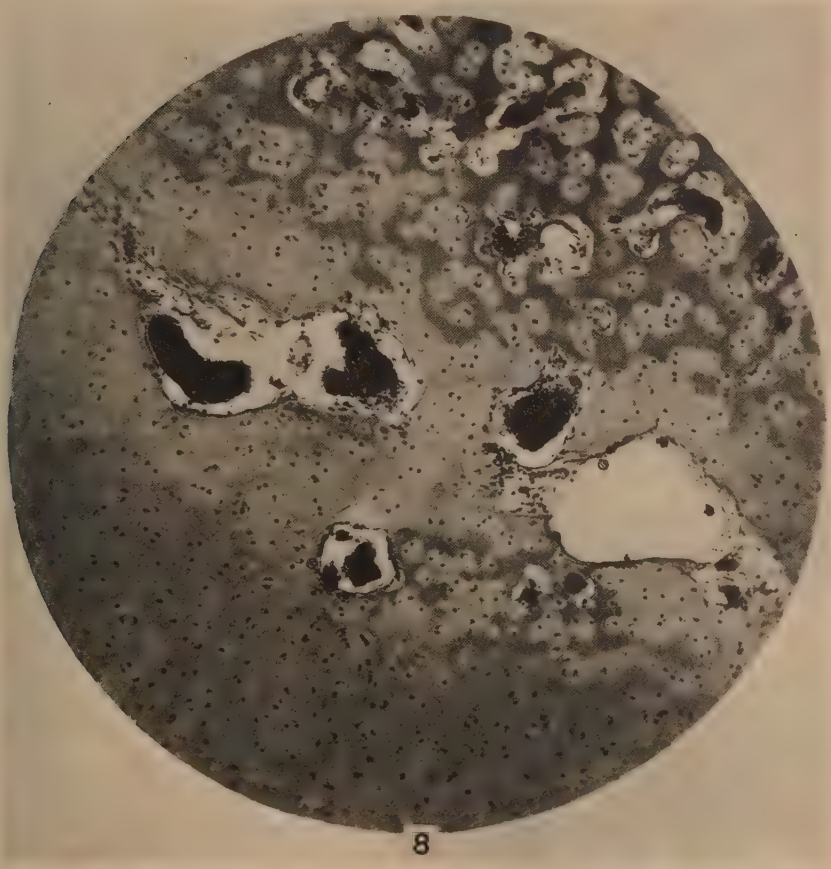


Fig. 8 This photomicrograph of the patella of a six-and-one-half-weeks-old fox-terrier puppy clearly illustrates the light orange-pink-staining zone between the blood vessel lining and the darker-appearing embryonic cellular cartilage. The cartilaginous matrix has little or no calcification, except where the process of ossification is more advanced. (Iron hematoxylin and eosin.) × 100.

cartilage and developing osteoid tissue (fig. 9). Where the ossification and calcification are more advanced in the center of primary ossification this dark festooned curve line is a calcified demarcation between the mesenchymal osteoblasts

Fig. 9 Photomicrograph of the center of ossification of a six-and-one-half-weeks-old fox-terrier puppy. At the center of ossification the dark festoon subendothelial line clearly is a demarcation between subendothelial osteoblasts and beginning calcification of the cartilage matrix. Many non-degenerated cartilage cells enter into the formation of the primitive osteoid tissue. (Iron hematoxylin and eosin.) × 750.

underlying the endothelium of the blood vessels on one hand and the deeper blue staining calcified cartilage matrix on the other.

In a seven-and-one-half-weeks-old fox-terrier puppy the fixed specimen of the patella is still smaller, actually, than those previously observed. Up to a certain stage of development, at this early period, the progressive condensation manifested by the development of the primary center of ossification is accompanied by an actual diminution, and not a relative diminution in the size of the patella (fig. 3). This patella measures 9.8 mm. in its long axis dorsoventrad and through the largest diameter cephalocaudad 4.5 mm. The largest amount of the primary center of ossification of the patella still underlies the zone of maximum convexity on the perichondrial aspect of the patella. The largest amount of the center of ossification immediately underlying the perichondrium is occupied by orange-pink-staining, vascularized, osteoid tissue (fig. 3). These are light places at the periphery of the center of ossification in the photomicrograph. Similar areas are seen at the periphery of the center of ossification toward the articular aspect (figs. 10 and 11).

THE DUPLEX ORIGIN OF THE OSTEOBLASTS FORMING THE PRIMARY BONE OF THE PATELLA

The subendothelial osteoblast is a modified mesenchymal cell. The unmodified mesenchyme by rapid proliferation fills in the gaps formed by the vacuolization of the cartilage cells. The modified cells of the mesenchyme assume in certain places an epithelioid appearance and are known as osteoblasts. The older cartilage cells having a broad intercellular matrix at the center of ossification undergo degeneration. All stages in cartilaginous nuclear changes, beginning with the pycnotic condition and continuing through the karyorrhectic to the complete karyolytic stages, are seen concomitant with the progressive vacuolization and liquefaction of the cytoplasm. These cells are destroyed.

There are younger cartilage cells, however, at the periphery of the center of ossification, which assume the typical characteristics of osteoblasts. These osteoblasts become incorpo-

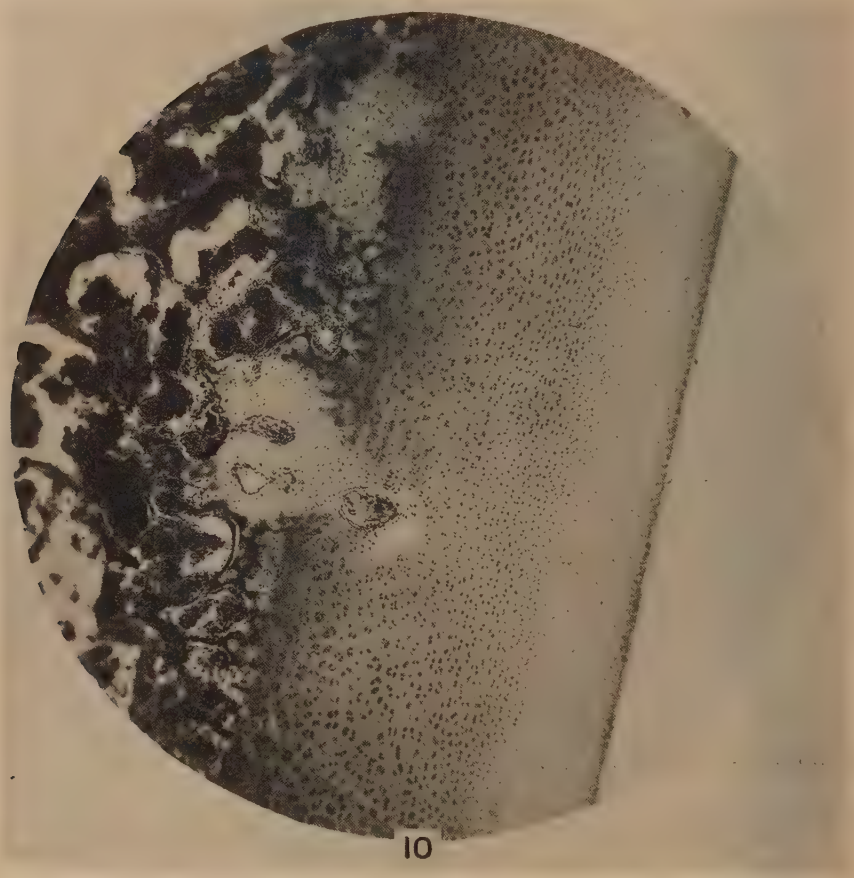


Fig. 10 Photomicrograph of the articular aspect of the center of ossification of the patella of a seven-and-one-half-weeks-old fox-terrier puppy, shown in figure 3. At the peripheral aspect of the center of ossification, illustrated in figure 3, there are orange-pink cartilaginous zones, forming bud-like projections, surrounding blood vessels. These orange-pink places are light in the photomicrograph. These osteoid orange-pink buds are recruited from cartilage cells of the embryonic type and from subendothelial osteoblasts. (Iron hematoxylin and eosin.) × 50.

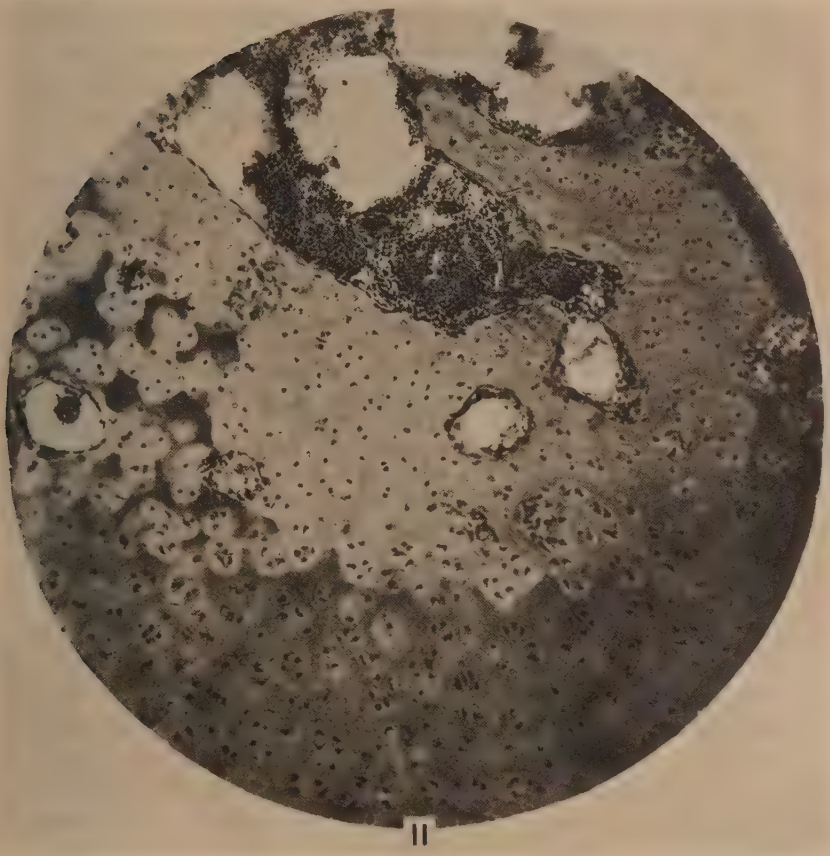


Fig. 11 Photomicrograph of an osteoid bud surrounding blood vessels, appearing light in the photomicrograph. This section is from the caudal aspect of the center of ossification at its periphery of the patella of a seven-and-one-half-weeks-old fox-terrier puppy, shown in figure 3. The cartilage cells entering into the osteoid trabeculae are not degenerated and the matrix is not calcified, but has all of the characters by which osteoid tissue is microscopically identified. In certain places at the periphery of the osteoid bud the cartilage matrix is calcified irregularly and appears dark on the photomicrograph. The transformation of primitive embryonic cartilage into osteoid tissue appears to be favored by close proximity to vascular channels—in other words, a close relationship to the source of nutrition. (Iron hematoxylin and eosin.) × 150.

rated into the primary bone and are indistinguishable as regards external form, internal structure, and staining reaction from young continuously related cartilage cells. These young cartilage cells develop a matrix which gradually assumes the characteristics possessed by osteoblasts, as regards staining reaction. The embryonic cartilage cells, however, gradually transform into bone cells similar to the changes that the subendothelial mesenchyme undergoes in the formation of bone cells (fig. 11).

In the later stages of development in the primary ossification of the patella there is more degeneration and less transformation of the cartilage cells into bone.

TRABECULAR ARRANGEMENT OF THE PRIMARY BONE OF THE PATELLA

In a nine-and-one-half-weeks-old fox-terrier puppy the dimensions of the fixed specimen of the patella are 11.8 mm. dorsoventrad and 5.5 mm. cephalocaudad through the largest diameter. In the midcephalocaudad section of the patella there are found two distinct arrangements of the bony trabeculae (fig. 4). Immediately underlying the periosteum on the ventrocephalic aspect of the patella the trabeculae are arranged dorsoventrad corresponding to the long axis of the patella. There are from four to eight trabeculae of bone in this location. At right angles to the former trabeculae there is a wider zone of bone, the bars of which extend cephalocaudad and have wider vascular channels than those existing between the subperiosteal trabeculae of bone. The intertrabecular vascular spaces immediately underlying the periosteum are narrow and elongated, corresponding to the long axis of the bony trabeculae. No cartilage is found immediately underlying the periosteum. There is a rim of cartilage which varies in width from 0.5 to 1.5 mm. on the caudal, dorsal, and cephalic aspects of the patella. The articular cartilaginous rim is approximately 1 mm. wide.

The subperiosteal bony trabeculae are directed along the lines of tension induced by the tendon of the quadriceps ex-

tensor muscle and its continuous patellar ligament. The trabeculae directed at right angles to the subperiosteal trabeculae are arranged along lines of pressure or compression, induced by the quadriceps extensor muscle and its ligaments where they pass over the pulley formed by the condylar end of the femur.

DISCUSSION

The origin of bone

There are three views with regard to the origin of bone:

1. That the osteoblasts are derived from the ectoderm, Geddes ('12).

2. That the osteoblasts are derived from the osteogenetic layer of the periosteum or from the subendothelial mesenchyme, Retterer ('05, '19), Fell ('25), Carey ('22), etc.

3. That osteoblasts may be derived in certain places by the direct transformation of young embryonic transitory cartilage, Van der Stricht ('90), Retterer ('00), Todd ('13), Macewen ('12), Gümbel ('06), Carey ('22), etc.

Brachet ('93) believes that the transitory cartilage after the liberation of cells from the matrix undergoes a regenerative process to form the marrow reticulum and that near the perichondrium these regenerated cells form osteoblasts and enter into the formation of bone tissue. The interpretation of whether cartilage cells do or do not form bone is dependent upon the material studied, and is not purely a question of fixation and staining reaction. If the process of ossification be studied in material which has a superabundance of large vesiculated transitory and older cartilage cells, then the interpretation of degeneration and complete disintegration of these cells is undoubtedly correct. The cartilage cells which show this degeneration are extremely large, and such cells of necessity must get into physiological difficulties of mechanical origin, for their mass increases as the cube, their surface, through which their content is nourished, as the square of their diameter. The limit of cell size is reached in these older transitory cartilage cells, and the center becomes

improperly oxygenated or nourished. The appearance of necrosis is directly due to the degree of hypertrophy of the cell.

In the cartilaginous patella there are many cells which never reach such a degree of hypertrophy as that found in the center of the diaphysis of the longer cartilaginous elements of the skeleton. These younger less differentiated transitory cartilage cells may become directly transformed into osteoblasts, the evidence of which is herein presented. Other areas have the large hypertrophied cartilage cells which do undergo dissolution. The fate of cartilage cells, therefore, is one of position and cannot be settled by the study of material from any one location. In certain positions the cartilage cells undergo extreme hypertrophy and disintegrate. In other positions the cartilage cell forms a less specialized scaffold, has undergone a less degree of cartilaginous differentiation, and may, under the proper environmental conditions, assume the attributes of an osteoblast.

The term cartilage, therefore, is a generic term, and there are many degrees in the development of hyaline cartilage. The amount and the density of the matrix will vary with age, and the degree of hypertrophy of the cell body likewise varies with the time element. If there be a gradual transformation of young embryonic cartilaginous cells into osteoblasts, there should be a gradual change observed in the morphology and staining reaction from one into the other. If there be no transformation, then a sharp line of demarcation between cartilage and bone will be presented.

CONCLUSIONS

1. The center of ossification of the primary bone of the cartilaginous patella begins beneath the summit of the convexity of the dense perichondrium, near the center of the cartilaginous patella.

2. Certain areas of the primary bone of the patella are formed by osteoblasts which have a duplex origin, namely, 1) subendothelial osteoblasts modified directly from the

mesenchyme surrounding the blood vessels and, 2) osteoblasts derived from young embryonic cellular cartilage closely related to blood vessels.

3. The arrangement of the trabeculae of the primary bone of the patella is in two directions, namely, 1) the subperiosteal bony trabeculae arranged in the long axis of traction of the tendon of the quadriceps extensor muscle; 2) pressure trabeculae directed at right angles to the subperiosteal tension trabeculae.

Acknowledgment is made to Mr. Leo Massopust, of the Department of Anatomy, for his aid in the preparation of the photomicrographs.

LITERATURE CITED

BRACHET, A. 1893 Étude sur la resorption du cartilage et le developpement des os longs chez les oiseaux. Monthly Internat. J. Anat. and Phys., T. 10, p. 391.

CAREY, E. J. 1922 Direct observations on the transformation of the mesenchyme in the thigh of the pig embryo (Sus scrofa), etc. Jour. Morph., vol. 37, no. 1, p. 1.

FELL, HONOR B. 1925 The histogenesis of cartilage and bone in the long bones of the embryonic fowl. Jour. Morph. and Phys., vol. 40, no. 3, p. 417.

GEDDES, A. G. 1913 The origin of the osteoblast and of the osteoclast. J. Anat. and Phys., vol. 47, p. 159.

GÜMBEL, T. 1906 Beitrag zur histologie des callus. Virchows Arch., Bd. 183, S. 470.

MACEWEN, WILLIAM 1912 The growth of bone, pp. 17, 18. Glasgow.

RETTERER, E. 1900 Evolution du cartilage transitoire. J. d'Anat. et de Phys., T. 36, p. 467.

———— 1905 Structure et histogenese de l'os. J. d'Anat. et de Phys., T. 41, p. 561.

———— 1919 Le processus de l'osteogenese varie selon les conditions locales et generales. C. R. Soc. de Biol., T. 82, p. 168.

VAN DER STRICHT, O. 1890 Recherches sur le cartilage articulaire des oiseaux. Arch. de Biol., T. 10, p. 1.

TODD, T. W. 1913 A preliminary communication on the development and growth of bone, etc. J. Anat. and Phys., vol. 47, p. 177.

THE LONGITUDINAL SMOOTH MUSCLE OF THE CENTRAL VEIN OF THE SUPRARENAL GLAND[1]

EARL F. HENDERSON

Fellow in Surgery, The Mayo Foundation, Rochester, Minnesota

SIX FIGURES

The circulation of the suprarenal gland originates in the three suprarenal arteries derived from the inferior phrenic artery, the aorta, and the renal artery, respectively. These enter the capsule of the gland and there give off many branches which supply a rich network of blood spaces between the columns of cells in the cortex. Through these spaces the blood passes inward to the larger sinus spaces of the medulla. The walls of these medullary sinuses consist of a layer of endothelial cells placed directly on the parenchymal tissue. The sinuses are connected with the central veins by small venous channels whose walls consist of little more than a layer of endothelial cells. Some of the sinuses themselves open directly into the central veins. Blood from these medullary sinuses is collected by the various branches of the central vein and discharged through the suprarenal vein into the inferior vena cava.

Within the walls of the central vein, immediately beneath the intima, are large bundles of smooth-muscle fibers running in a longitudinal direction. Circular bundles are not found.

One of the earliest and probably the best description of this peculiar musculature was given by von Brunn ('73). He noted that these muscle bundles accompany practically all

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery, December, 1926. Submitted for publication April 19, 1927.

veins 0.2 mm. or more in diameter and that they are never missing from the division points of veins of this size, where they fill in the angle between the two branches. Gottschau ('83) mentioned the occurrence of these muscle bundles, and Guarnieri and Magini ('88) spoke of them as large fasciculi of smooth muscles which surrounded the veins as strong belts. Flint ('00) stated that he had observed these muscle bundles in the veins of the medulla, but Ferguson ('06) made the first real attempt in American literature to describe them. He suggested that they might have some close physiologic relation to the function of the gland, without, however, advancing any hypothesis to explain what this relationship might be.

Lichtwitz ('08) and Kolmer ('18) also commented on this peculiar musculature, but the first noteworthy attempt to explain its possible mode of action was made by Kutschera-Aichbergen ('22). He called attention again to the delicate endothelial tubes with scarcely any demonstrable wall, which open into the central vein, and suggested that contraction of these muscle bundles might produce constriction of the openings of these thin-walled tributaries, resulting in a damming back of the blood in the medulla and cortex. As the blood could not leave by way of the central vein, it would overflow through the capsular veins and again reach the general circulation, thus producing a closed system of vessels in the gland extending inward as far as the central vein. He therefore concluded that this longitudinal musculature would serve to effect a division of the blood charged with epinephrin, determining how much of it would pass out through the central vein and how much would be deflected back through the capsular veins.

In order to formulate an intelligent conception with regard to the physiologic significance of this muscular mechanism, it seemed necessary to determine more accurately the course and distribution of the bundles. Serial sections of a suprarenal were therefore made, and from these the main trunk of the central vein was reconstructed in wax. To facilitate fixing and cutting, a rather small, thin gland was selected for

this purpose. The sections were cut 16 μ thick, and every other one was used for reconstruction. The wax plates were 2 mm. thick, giving a longitudinal magnification of 62 times. The lateral magnification was, of course, made to conform with this so that the proper proportions were maintained. The wax plates were made of a mixture of six parts beeswax, four parts paraffin, and two parts resin. A careful study was also made of cross-sections of the central veins of the suprarenal glands from 176 consecutive necropsy specimens.

Longitudinal fasciculi were found within the walls of the main trunk of the central vein throughout almost its entire length and within the walls of its main branches. They were situated immediately beneath the intima. In no instance were circular bundles observed. In the gland used for serial sections and reconstruction, definite well-defined muscle bundles were beginning in the main trunk of the central vein at a point 0.5 cm. from the distal end, or at a point whose distance from the distal end of the gland was equal to about one-thirteenth of the total length of the gland.

In the smaller veins the fasciculi are usually single, rounded, and often apparently project far into the lumen of the vessel. As the veins become larger, two or three or more of these bundles may be found arranged irregularly on the vessel's circumference. They are still more or less rounded in shape and produce marked thickenings in the vessel walls. Often they divide to permit the entrance of one of the medullary sinuses, or one of the thin-walled vessels connecting the medullary sinuses with the central vein. These thin-walled tributaries also pierce the thinner portions of the wall between the longitudinal fasciculi. As the bundles become larger and more numerous they encroach more and more on one another, as a result of which they lose their rounded contour, become flattened, and are more uniformly distributed around the circumference of the vessels.

Before emerging from the gland, the main trunk of the central vein is a thick-walled vessel consisting of an endothelial intima, a strong coat of longitudinal smooth-muscle

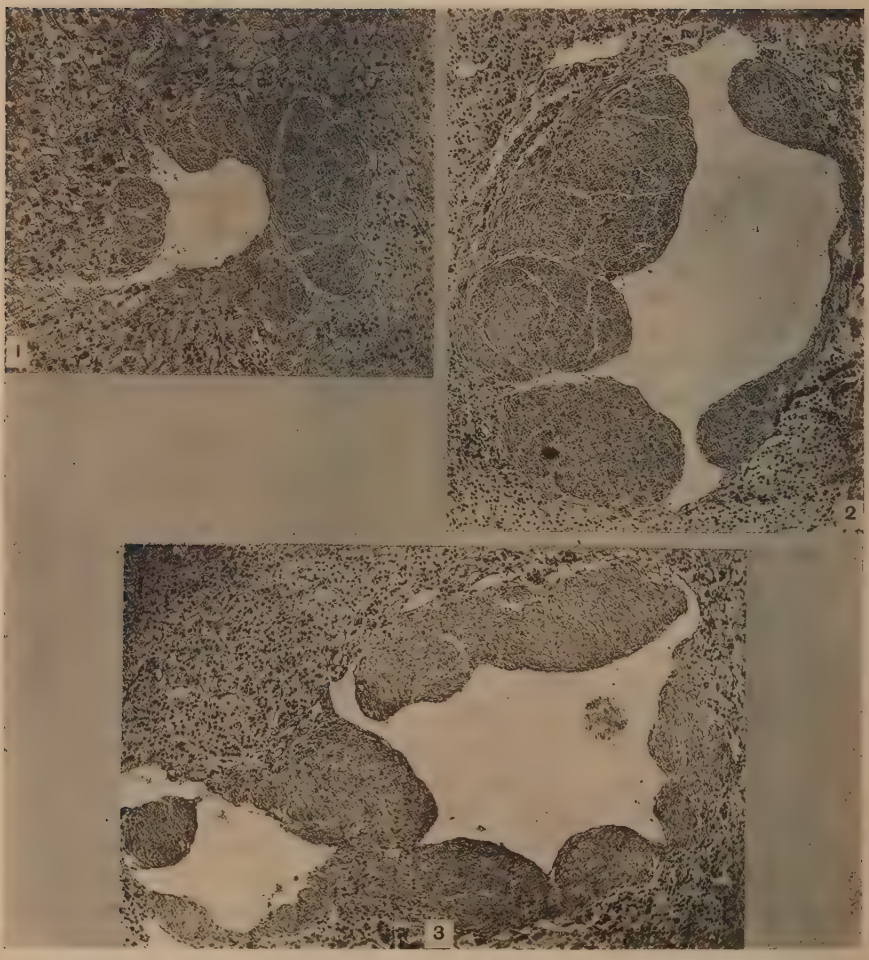


Fig. 1 Longitudinal muscle bundles in the wall of a central vein. Two thin-walled venous tributaries may be seen entering this vein. × 80.

Fig 2 Heavy longitudinal bundles situated principally on one side of a central vein. These bundles are immediately beneath the intima. × 80.

Fig. 3 A peculiar arrangement of longitudinal muscle in the wall of a central vein and one of its tributaries. × 60.

fibers arranged quite uniformly about its circumference, and connective-tissue adventitia. Figures 1 to 3 show in cross-section some of the more striking forms of this musculature.

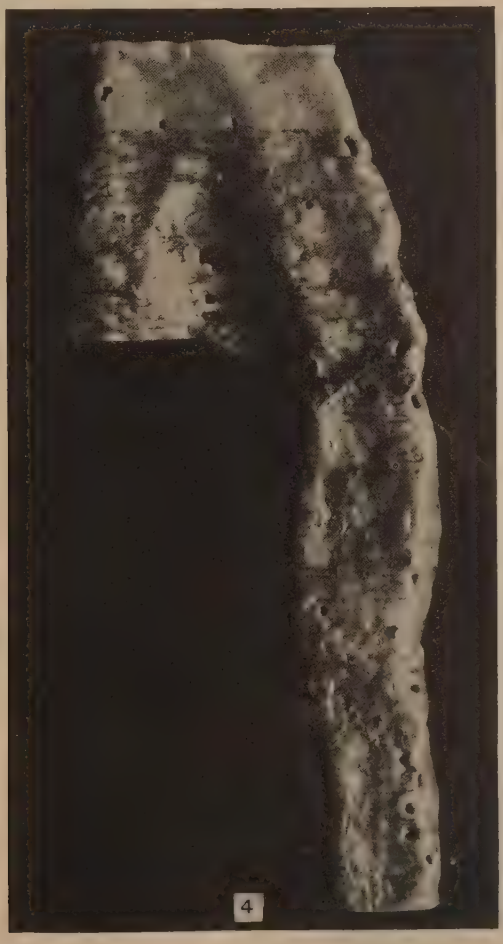


Fig. 4 Reconstruction in wax of a portion of a central vein beginning near the distal end of the gland. The ridge of longitudinal muscle in each of the limbs of the vein may be noted. The openings mark the points of entrance of thin-walled venules or sinuses.

A study of the course of these longitudinal bundles, as shown in the models made from serial sections (figs. 4 and 5), yields some interesting information. Model 1 (fig. 4) shows a reconstruction of the first portion of the vein, beginning at a point 0.5 cm. from the distal end of the gland. A well-defined muscle bundle, shown as a prominent ridge on the model, is seen coursing along the left side of the longer of the two branches of the model. A similar bundle is seen arising in the wall of the left branch of the vein. As the two branches of the vein unite, these two muscle bundles come together and fuse in the angle of union. That these muscle fibers are not continued along the course of the vein is seen in model *a* (fig. 5), which is a direct continuation upward of model *1*, though shown at a reduced magnification. This is in accord with the early observations of von Brunn, who described these bundles as occurring first in the smaller vessels at the point of union of two of these vessels, where they filled in the angle between the two anastomosing branches. The small openings in the model mark the points of entrance of the thin-walled vessels such as are seen in figure 1. It is significant that most of these small vessels pierce the wall of the central vein where it is very thin and do not come in contact with the longitudinal bundles. It is worthy of note, too, in view of Kutschera-Aichbergen's theory, that many more of these small tributaries enter the distal branches of the central vein, where the walls are thin and the longitudinal muscle bundles are rather poorly developed, than enter the larger trunks where the bundles are so much larger.

Model *a* (fig. 5) shows some small bundles (seen as rounded thickenings of the wall in the left-hand model) on the extreme left side of the vessel. The models on the right (fig. 5) represent the reconstruction of the segments of the lumen of the vessel shown on the left; the small projections, the tributaries which have pierced the wall of the vessel. In the reconstruction of each of the remaining models (*b* to *k*, inclusive) every other one of thirty consecutive sections were used, and the next ninety were discarded. Models *b* and *c*

Fig. 5 Reconstruction of segments of the same central vein used in the preceding model (fig. 4). The course of the longitudinal muscle bundles is shown. The models on the right are reconstructions of the lumen of the vein.

show the longitudinal muscle grouped at the left side of the vessel and in each case a small tributary piercing the wall on this side. In model *d* a number of muscle bundles are situated at various points in the circumference of the vessel, the principal ones, however, still being on the left side. A somewhat larger tributary is seen piercing the wall of the vein diagonally, as shown by the model on the right.

Model *e* shows that, as this vein is approaching anastomosis with another vein, the longitudinal muscle bundles have been massed on opposite sides of the vessel. A good-sized bundle may be seen in the angle between the vessels and another large mass on the extreme left side. The other portions of the wall are relatively thin. Model *f* shows these two veins at the point of anastomosis. The sections show that there are a few longitudinal bundles in the median portion of the vessel continuing from the angle of union, as shown by the thickening of the wall in this portion of the model. There are also a few longitudinal fibers throughout the main portion of the circumference of this model. The heavy longitudinal bundle, however, is still found on the left side. This is a continuation of the heavy bundle seen in model *e*. The model on the extreme left shows a smaller vein approaching the main trunk. Here again the longitudinal muscle is massed on the side of the vessel nearest the main trunk. Model *g* shows the anastomosis of these two vessels. The heavy bundles on the adjacent sides of the approaching vessels have fused at the angle of anastomosis, while at the extreme ends other heavy longitudinal masses have appeared.

In model *h* the vessel has lost some of its flattened appearance after passing the point of anastomosis. There is a certain amount of longitudinal thickening at various points in the circumference, but the main portions are still massed on opposite sides of the vessel. Model *i* shows these longitudinal fasciculi in much the same relative positions. A smaller thin-walled vessel is seen coming in from the right. Models *j* and *k* show sections of the same vein farther along in its course. In these the longitudinal bundles are somewhat more uniformly distributed around the circumference of the vessel.

From this study it would seem that as two small veins, each of which has only one longitudinal muscle bundle, approach the point of anastomosis, these bundles occur on adjacent sides of the vessels, and when the vessels unite these muscles fuse in the angle of anastomosis. As two larger veins approach the point of anastomosis, the longitudinal fibers

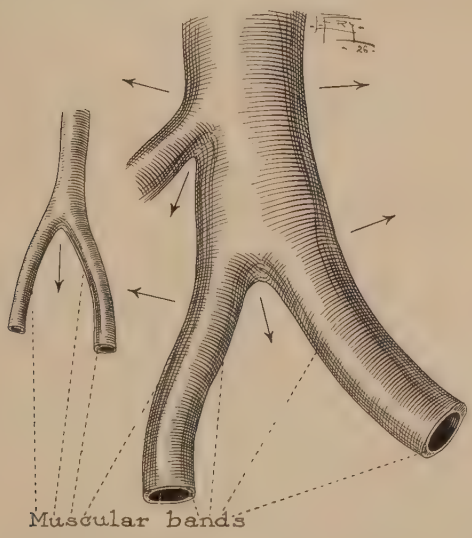


Fig. 6 Diagram of the arrangement of the longitudinal musculature in the central vein of the suprarenal. These figures show that when two veins anastomose, the bundles on the adjacent sides fuse in the angle of union. As shown in the diagram, contraction of this musculature would produce active dilatation of the vein.

in each arrange themselves for the most part in two main masses, one on the side nearest the approaching vessel, and the other on the opposite side. When these two vessels join, the muscle bundles on the adjacent sides of the two fuse in the angle and the bundles on the opposite sides continue along the outer wall of the vessel past the point of anastomosis. This arrangement is shown diagramatically in figure 6.

The significance of this peculiar muscular arrangement in the wall of the central veins of the suprarenal gland probably consists in the fact that contraction of the muscles results in active dilatation of these veins. We may, therefore, expect that this mechanism regulates the outflow of blood and consequently epinephrin stored in the medulla. There is undoubtedly a rapid discharge of epinephrin in times of stress which is probably controlled by the sympathetic nerves through the splanchnic nerves.

In view of this hypothesis regarding the mode of action of the peculiar musculature of the central vein, it seems reasonable to conclude that the discharge of epinephrin is in part regulated by the action of the muscles and in part by the rate of secretion of the gland itself.

BIBLIOGRAPHY

VON BRUNN, A. 1873 Ueber das Vorkommen organischer Muskelfasern in den Nebennieren. Nachr. v. d. k. Gesellsch. d. Wissensch., pp. 421–423.

FERGUSON, J. S. 1906 The veins of the adrenal. Am. Jour. Anat., vol. 5, pp. 63–71.

FLINT, J. M. 1900 The blood vessels, angiogenesis, organogenesis, reticulum, and histology of the adrenal. Johns Hopkins Hosp. Rep., vol. 9, pp. 153–228.

GOTTSCHAU, M. 1883 Structure und embryonale Entwickelung der Nebennieren bei Säugethieren. Arch. f. Anat., S. 412–458.

GUARNIERI, G., AND MAGINI, G. 1888 Études sur la fine structure des capsules surrénales. Arch. ital. d. biol., vol. 10, pp. 379–384.

KOLMER, W. 1918 Zur vergleichenden Histologie, Zytologie, und Entwicklungsgeschichte der Säugenebenniere. Arch. f. mikr. Anat., Bd. 91, S. 1–139.

KUTSCHERA-AICHBERGEN, HANS 1922 Nebennierenstudien. Frankfurter Ztschr. f. Path., Bd. 28, S. 262–294.

LICHTWITZ, L. 1908 Über einen Fall von Sklerodermie und Morbus Addisonii nebst Bemerkungen über die Physiologie und Pathologie des Sympathicus und der Nebennieren. Deutsch. Arch. f. klin. Med., Bd. 94, S. 567–587.

THE RETICULUM OF THE HUMAN SPLEEN

NATHAN CHANDLER FOOT
Department of Pathology, College of Medicine, University of Cincinnati, Cincinnati, Ohio

TWO PLATES (FOUR FIGURES)

INTRODUCTION

Judging from statements in modern text-books of histology and in the current literature, very little progress has been made in coming to definite conclusions as to the exact histology of the splenic reticulum; some writers describe it as generally distributed, while others deny its existence outside the capsule or trabeculae. We read on page 262 of Bailey's Text-Book of Histology ('26) a quotation from Mollier's article of 1910, in which the venous sinuses are described as being composed of a protoplasmic endothelial syncytium, outside of which lies a plexus of closely arranged longitudinal reticulin fibrils which overlie the lengthwise-running strands of protoplasmic reticulum; outside of these are circular fibers of reticulin which are continuous with those of the splenic pulp, and, finally, between the protoplasmic and reticulin fibrils is interposed a thin, structureless, and fenestrated membrane. Stöhr's Lehrbuch der Histologie ('10) describes the structure of the sinuses in very similar terms, merely mentioning the membrane as supporting the endothelial cells and indicating that it is lacking in the intralobular veins. The later English editions of this book have not materially altered this description to date. Stöhr makes two hypothetical statements in passing: he indicates that the circular fibrils probably serve to keep the veins open and are presumably composed of reticulin rather than elastin, and he states that

the protoplasmic, rod-like prolongations of the endothelial cells are probably contractile.

Robinson ('26), writing very recently, quotes Mall to the effect that the fenestrated 'barrel-stave' endothelium is limited to the intralobular veins and their radicles and is not found in the walls of the venous sinuses. He states that he was unable to demonstrate any reticulum in the pulp of perfused spleens, this being composed of stellate, anastomotic cells whose protoplasmic processes extend outward in long, filamentous prolongations to form the sponge-like network that supports the pulp; "What appears to be reticulum," he says, "is merely the protoplasmic processes of pulp cells." He quotes Weidenreich, who claimed that the annular fibrils, as well as those of the pulp, were protoplasmic rather than reticular in nature. Thus we have two diametrically opposed views which should be reconciled. We shall discuss this later.

Those text-books of pathology devoting a section to the normal histology of the spleen do not differ from those on histology proper; MacCallum ('21) cites Mollier at length; Schridde ('23), in Aschoff's Pathologische Anatomie, describes essentially the same architecture, adding to it a very delicate reticulum of minute fibrils that lie between the endothelium and the membrane of the venous sinuses and, as he says on page 141, are best seen in sections stained by the Altmann-Schridde method. Mallory ('23), in his Principles of Pathologic Histology, gives an excellent description of the micro-anatomy of the spleen, mentioning the points already noted, but omitting all mention of the membrane. He calls attention to the normally insignificant amount of pulp tissue between the venous sinuses—a point insufficiently stressed by other writers. He does not specifically mention reticulum by name, but his description of the fibrils surrounding the venous channels tallies in every respect with that of the earlier writers; at all events, he does not describe them as being protoplasmic.

Thus the literature is fairly well in accord as to the presence of a fenestrated, sieve-like endothelium composed of

elongated cells and lining the walls of the veins and venous sinuses. The majority of the writers favors a surrounding network of reticulin fibrils; the only serious difference of opinion concerns itself with the presence or absence of the delicate, fenestrated, structureless membrane between these and the endothelium. The precise distribution of the reticulum of the organ is usually sketched in an inconclusive way, as though the authors hesitated to commit themselves on this point.

In order to rectify this impression and, if possible, to fill the hiatus in our knowledge as to the exact distribution of the splenic reticulum, a careful study of a large number of human spleens, removed at necropsy, has been undertaken. By using three somewhat different methods of silver impregnation and a number of familiar stains, it has been possible to arrive at definite conclusions.

TECHNIQUE

Human spleens were removed at necropsy one to twenty-four hours postmortem, and were sectioned and fixed in Zenker's fluid, or they were perfused for varying periods of time (at a controlled pressure of 90 to 100 mm. of mercury) with normal saline solution and then fixed by injecting either Zenker's fluid or 10 per cent neutral formalin through the perfusion cannula into the splenic artery. The effluent from the splenic vein was collected at the hilum in these cases, smeared on slides, air-dried, and stained with Wright's stain to determine what cells were being washed out. It was found, even in cases of acute infection, that from 60 to 80 per cent of these were large mononuclear and the rest chiefly lymphocytes.

Paraffin sections were cut at thicknesses varying from 7 μ to 28 μ and either stained or impregnated by the following methods: stains—Harris hematoxylin-eosin, Heidenhain's iron-hematoxylin, Mallory's phosphotungstic acid hematoxylin; impregnations—modifications of Bielschowsky's silver-ammonium oxid (Foot, '24), Hortega's silver-ammonium car-

bonate (Foot and Mènard, '27), and Achúcarro's silver-tannate method (Foot, '27).

RESULTS OF OBSERVATIONS

The splenic reticulum is very clearly and precisely demonstrable by these methods and is found to have the distribution that will be described presently. It is to be noted that by 'reticulum' is meant a fibrillar connective tissue composed of delicate strands that become specifically impregnated with silver and are either composed of, or combined with, a substance known as 'reticulin.' Some fibrils show more affinity for silver than do others, but one is forced for the present to include under the term 'reticulum' all those that become definitely impregnated by silver solutions.

Capsule and trabeculae

The reticulum here is coarse, straight, and intimately intermingled with elastic, fibrous, and very scanty smooth muscular tissue; reticular fibers are distributed more copiously on the inner aspect of the capsule and the margins of the trabeculae than they are in their deeper portions. Delicate fibrils enter or leave the capsule or its prolongations to become continuous with those of the pulp, but there is no indication that the latter are derived from the coarser capsule, they are merely continuous therewith.

Arteries

The larger arteries have the usual appearance of arteries in general and their reticulum is limited to the media and adventitia, leaving the intima more or less isolated. It runs in concentric strands. The smaller sheathed arteries, however, are enmeshed in a delicate and very complicated network of reticulum that anastomoses repeatedly and encloses the vessels in a web of fine fibrils that may run in any or all directions (fig. 1). The terminal arterial capillaries possess a very delicate reticulum resembling that of capillaries in

general, the ampullae of Thoma (which are essentially merely bulbous terminal capillaries) have a similar delicate network of reticulin fibrils, scanty and rather wide-meshed.

Periarterial organs

The reticulum of the malpighian corpuscles (fig. 2) is quite similar to that of the lymph nodes; there is a coarse mass about the central arteriole, continuous with the midzonal reticulum; the latter is more delicate and is arranged in rounded alveolar strands that enclose masses of lymphoid tissue; the peripheral reticulum is, again, rather coarse and compact. There seems to be a concentric arrangement of the coarser fibers, so that the structure may be likened to a spider web, whose meshes are gently rounded, rather than rectilinear. The amount of reticulum in the splenic corpuscles varies a good deal according to the age of the subject, the mechanical conditions of the circulation, etc. Some corpuscles have a good deal, while others show very little; youth and venous congestion seem to favor an abundance of this tissue.

The reticulum of the ellipsoids (fig. 3) is continuous with that of the pulp and neighboring vessels, but it is extremely delicate and its arrangement very complex where it penetrates among the cells and forms a sort of felting.

Veins

The larger veins have reticular sheaths that differ in no wise from those of other veins, but the intralobular vessels and their radicles have a peculiar structure and their reticulum is likewise quite distinctive (fig. 3). These vessels are composed of elongated endothelial cells which Robinson ('26) has compared with barrel staves, separated from one another by spaces, as though the barrel had been partially unhooped. These cells are surrounded by annular reticulum fibrils that run at right angles to the long axis of the vessel (or roughly so) and form approximately parallel rings. On cross-section,

they are seen to be interrupted at intervals and to be continuous with the pulp reticulum, leaving it on one side of the vessel to unite with that on the opposite side. They anastomose sparingly with one another, branching more or less (fig. 3); often pulp fibrils coalesce to form such annular fibers which, in turn, fan out upon leaving the vein on the opposite side, to become several pulp fibrils. There are no definitely continuous longitudinal reticulin fibers in direct apposition to the vessel walls, although coarse trunk fibrils do run longitudinally in the pulp at a short distance therefrom and give off the annular fibrils at intervals (fig. 3). As already stated, Mollier has described longitudinal fibrils of reticulum lying along the endothelial rods; these can be readily demonstrated with silver tannate or phosphotungstic acid hematoxylin, but they are brownish and not black with the former and blue and not yellowish-brown with the latter, so that they are probably not reticulin, but some other substance. Furthermore, they are definitely part of the endothelial cells, rather than an outside, supporting structure. They will be discussed at length in a subsequent paper.

The annular fibrils show a marked variability in their affinity for silver, more so than do those of the pulp or the arteries; they may prove to be quite refractory to impregnation with silver-ammonium oxide or carbonate and, in that case, require silver tannate for satisfactory demonstration. They stain with eosin, iron hematoxylin, and acid fuchsin; they are golden yellow to brownish in phosphotungstic acid hematoxylin preparations. Perfusing the spleen with salt solution seems to lessen the argyrophilia of these fibrils quite materially, which might suggest a certain degree of solubility on the part of the reticulin in saline solution, were it not for the fact that silver tannate will demonstrate that it is still present. It is quite possible that the sodium chloride is to blame for this phenomenon.

Venous sinuses

These vascular channels form the greater part of what is usually known in a broad sense as 'the splenic pulp'; they are dilatations of the venous system and are composed of an endothelium that varies in its structure: where the sinuses debouch into the radicles of the intralobular veins, the endothelium is of the barrel-stave variety, but the fenestra are wider and the cells converge as the lumen narrows to form the venous radicle. Where the sinuses are dilated to their maximum caliber, the endothelium becomes more anastomotic, the cells no longer run parallel, but their processes run in all directions. Although the endothelium differs somewhat from that of the intralobular veins, the reticulum is essentially the same in its arrangement, as can be clearly seen in figure 4, where the field is almost entirely made up of venous sinuses.

Pulp

The supporting structure of the pulp is composed of stellate, anastomotic 'reticulo-endothelial' cells intimately overlaid by a rather coarse, irregularly distributed reticulum that becomes deeply impregnated with silver and appears to partake largely of the appearance of lymphoid reticulum. This is best shown in the spaces between the sinuses in figures 3 and 4. That it exists as an argyrophil reticulum is readily demonstrable, provided one employ suitable technic. This reticulum, though rather coarse, apparently has relatively little tensile strength, for it becomes readily broken up into fine tangles and snarls in the case of splenic engorgement.

SUMMARY

Having considered the distribution and appearance of the splenic reticulum at length, we may now sum up our conclusions as follows:

1. The splenic reticulum may best be demonstrated by silver impregnations of paraffin sections; perfusing the organ somewhat interferes with successful impregnation, hence more

than one method should be employed before definite conclusions can be drawn as to the histology of the reticular tissue.

2. The reticulum is everywhere continuous, but its arrangement differs with its situation: *a*) In the capsule and trabeculae it is coarse and straight. *b*) Around the arterioles it is complex and web- or felt-like, as it is in the ellipsoids. *c*) In the splenic pulp and corpuscles it is indistinguishable from lymphoid reticulum. *d*) Where it surrounds the intralobular veins, their radicles and the venous sinuses, it encloses them in annular fibrils that are quite distinctive. These do not form unbroken rings, but anastomose sparingly and are frequently interrupted in their course.

3. There are no coarse, parallel reticulum fibers in the walls of the veins or venous sinuses; apparently the non-reticular fibers in the endothelial cells or the longitudinal fibers of the neighboring pulp have been mistaken for part of the venous reticulum.

The assistance of Mr. Marvin C. Mènard, who helped in the collection, selection, and technical preparation of the material to be studied, and of Mr. J. B. Homan, who finished the photomicrographs, is most gratefully acknowledged.

LITERATURE CITED

BAILEY 1926 Bailey's text-book of histology, O. S. Strong and A. Elwyn, 7th ed., p. 258. New York.

FOOT, N. C. 1924 A technic for demonstrating reticulum fibers in Zenker-fixed paraffin sections. J. Lab. and Clin. Med., vol. 9, p. 777.

———— 1927 A silver tannate technic for paraffin sections from the central nervous system. Arch. Path., vol. 4, p. 36.

FOOT, N. C., AND MÈNARD, M. C. 1927 A rapid method for the silver impregnation of reticulum. Arch. Path. (In press.)

MACCALLUM, W. G. 1921 A text-book of pathology, 2nd ed., p. 802. Philadelphia and London.

MALLORY, F. B. 1923 Principles of pathologic histology, p. 610. Philadelphia and London.

ROBINSON, W. L. 1926 The vascular mechanism of the spleen. Am. J. Path., vol. 2, p. 341.

SCHRIDDE, H. 1923 In Aschoff's Pathologische Anatomie, 6th ed., Bd. 2, S. 140. Jena.

STÖHR, P. 1910 Lehrbuch der Histologie und der mikroskopischen Anatomie des Menschen, 14th ed., p. 132. Jena.

PLATES

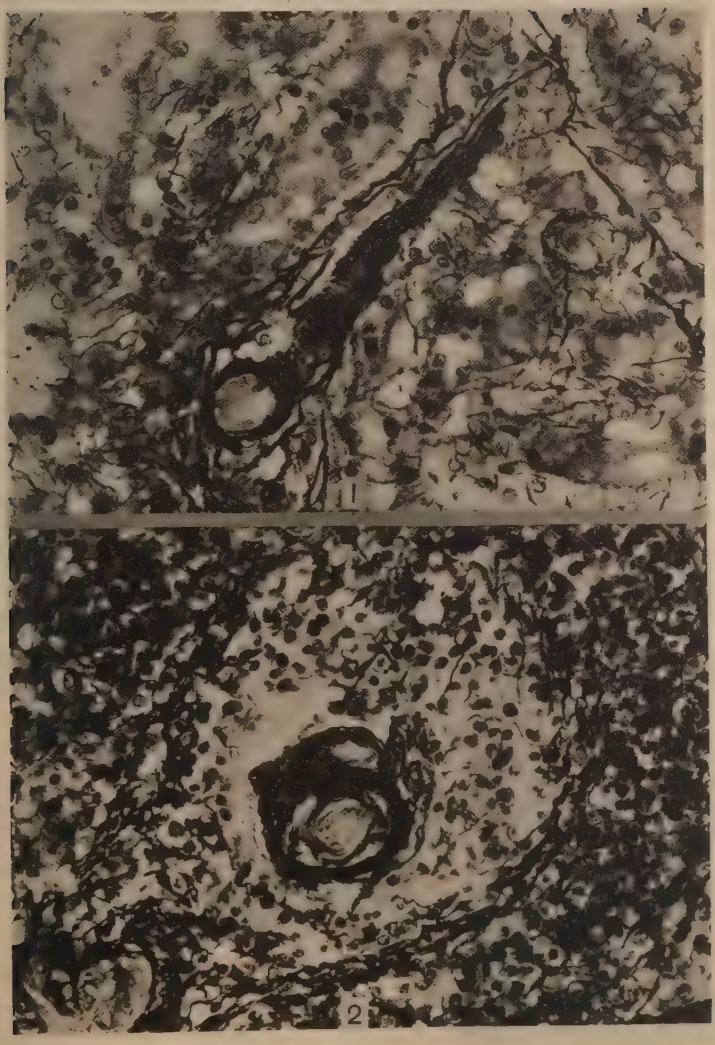
PLATE 1

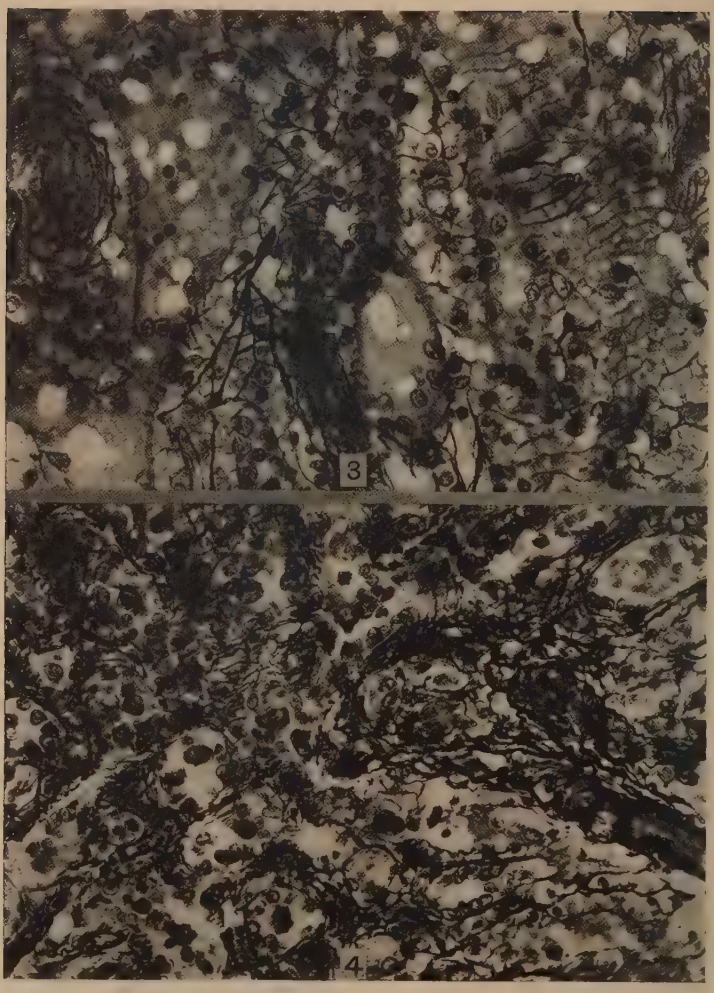
EXPLANATION OF FIGURES

All these photomicrographs were made with a 4-mm. Leitz objective and a magnification of approximately 500 diameters, reduced by one-third for reproduction. The impregnations were silver tannate in all but figure 3, which was a silver-ammonium carbonate; the counterstain was Van Gieson's stain.

1 An arteriole and its reticular sheath, surrounded by venous sinuses. Note the marked difference in the two types of reticulum, also the severed ends of the annular fibrils of the sinuses.

2 The reticulum of a malpighian, or splenic corpuscle.


1
2



3 An arteriole and an ellipsoid are seen, with their complex reticulum. At one side are several intralobular veins, the annular fibrils and barrel-stave endothelium of which are plainly visible. Note also the coarse pulp reticulum near the constricted sinus between the arteriole and the ellipsoid.

4 Chiefly venous sinuses, with an artery at one side. Note how little pulp there is between the sinuses.

ON THE ENDOTHELIUM OF THE VENOUS SINUSES OF THE HUMAN SPLEEN

NATHAN CHANDLER FOOT

Department of Pathology, College of Medicine, University of Cincinnati, Cincinnati, Ohio

TWELVE FIGURES

INTRODUCTION

Although it has been recognized since the time of Malpighi that the endothelium of the splenic veins and their tributaries has a special morphology, differing greatly from that of vascular endothelium in general, there is still much doubt as to its exact arrangement and architecture, and there are, therefore, marked discrepancies in the text-book descriptions of this tissue (Bailey, '26; MacCallum, '21; Mallory, '23, Schridde, '23, and Stöhr, '24). It is certain that this endothelium is composed of rod-shaped cells, whose nuclei bulge into the lumina of the vessels; that they run approximately in the long axis of the veins, with spaces or fenestra between them, and that there are indications that they may be contractile. Doubt exists as to their having such an arrangement in the sinuses, Mall (Robinson, '26) having claimed that they ran a parallel course only in the intralobular veins and their radicles. Stöhr ('24) describes the sinus wall as a grid-like plasmodium composed of elongated cells that represent the longitudinal members of the grid and transverse members that connect the cells with one another, in the fashion of intercellular bridges. He states that, in man, these transverse processes have atrophied to become almost entirely transformed into supporting fibers ('Ringfasern') which are everywhere closely apposed to the reticulum fibers of the pulp. Szymonowicz ('24) gives a similar description, but specifies

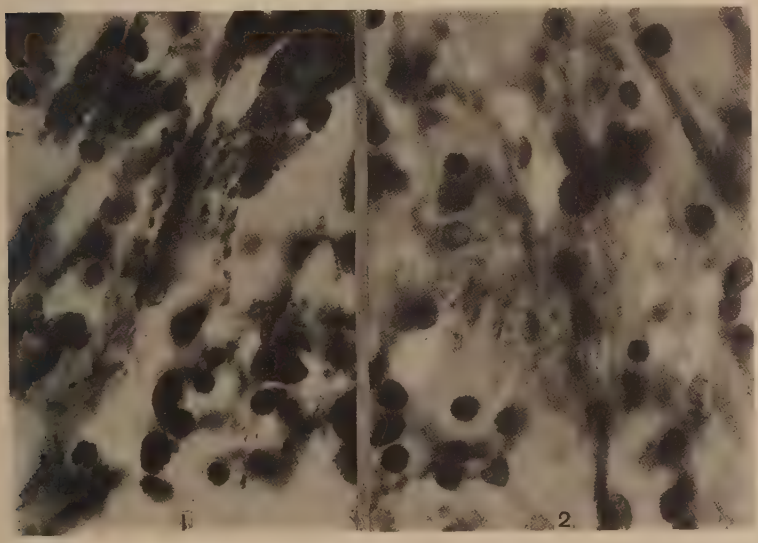
that the basal portion of the elongated endothelial cells show rod-like fibrils (on cross-section) which are superimposed upon the cytoplasm and represent the longitudinal strips that bulge outward and, where the sinus wall is caught tangentially, appear as thickly crowded longitudinal fibers. In isolated endothelial cells he has observed regularly distributed bands, or transverse wheals, that suggest the possibility of contractile organs.

The question as to the arrangement of the reticulum that surrounds the venous system in the spleen has already been discussed in a previous paper (Foot, '27). Mollier ('10) and Schridde ('23) describe a fenestrated, structureless membrane upon which the endothelial cells rest. Mallory ('23) omits all mention of it entirely. Thiel and Downey ('21) discuss it at length. Furthermore, Schridde ('23) mentions a delicate, anastomotic, protoplasmic plexus of fibrils that lie between this membrane and the endothelium. Thus we have conflicting statements that cast doubt upon the veracity of the accepted conceptions of splenic micro-anatomy.

While experimenting with this organ and endeavoring to impregnate the reticulum in perfused specimens (Foot, '27), a modification of the Achúcarro silver-tannate method was resorted to, with good success so far as the reticulum was concerned and with rather startling results in the impregnation of the endothelium. The cells of the venous sinuses, intralobular veins and their radicles were everywhere crossed by dark bands that suggested the striae in skeletal muscle (fig. 1). Barely visible in eosin-stained sections, they were just demonstrable with Heidenhain's iron hematoxylin, but were most strikingly brought out in sections stained with Mallory's phosphotungstic hematoxylin. Further study of these bands completely confirmed Szymonowicz's description of them before the latter had come to my attention.

These facts are the basis for this discussion; we must decide upon a number of questions and try to arrive at definite conclusions as to the true structure of the venous endothelium of the spleen. The points to be settled are: *a*) Are there

intercellular bridges, as Stöhr intimates? *b*) Is the endothelium of the sinuses very different from that of the intralobular veins and their radicles? *c*) Is there a structureless



All drawings made with an Abbe-Zeiss camera lucida, which was used to block in the outlines, the details being subsequently filled in free-hand. The photomicrographs were made with the assistance of Mr. J. B. Homan, of the Department of Medical Art. Mr. M. C. Mènard rendered valuable technical assistance in the preparation of the sections.

Fig. 1 Oil-immersion photomicrograph (× 2000, reduced one half) of a section of the spleen impregnated with silver tannate after perfusion with normal saline solution. The bands in the endothelium are well shown.

Fig. 2 Oil-immersion photomicrograph (× 1500, reduced one half) to confirm figure 7. Note the pale 'hoops' and the H-like arrangement of the darkly staining material in the endothelial bands. Same stain.

fenestrated membrane between this and the reticulum? *d*) Is there a delicate protoplasmic network between it and that tissue? *e*) What is the nature of the striations observed in phosphotungstic acid hematoxylin and silver-tannate preparations?

MATERIAL AND TECHNIQUE

The material used was all obtained from human subjects at necropsy, from a few minutes to several hours' postmortem. Some spleens were immediately fixed in Zenker's fluid, others were perfused with normal saline solution at room temperature for varying lengths of time. The pressure was maintained at 100 mm. mercury and carefully checked up with a manometer. The perfused spleens were fixed by injecting either Zenker's solution, or 10 per cent neutral formalin into the splenic artery under moderate pressure. Fresh smears were taken from a spleen that was still warm and were fixed in Zenker's solution or 10 per cent neutral formalin; at the same time hanging-drop preparations of scraped spleen were examined in a drop of normal saline solution in a warm chamber under the microscope.

The study of the endothelium was focused chiefly upon sections from spleens removed less than an hour postmortem and proved to be still viable. The material was cut in paraffin at varying thicknesses, serial sections being used in some instances. The sections were stained with hematoxylin-eosin, Heidenhain's iron hematoxylin, Van Gieson's stain, and Mallory's phosphotungstic acid hematoxylin; they were impregnated by the silver-ammonium carbonate (Foot and Mènard, '27) or silver tannate (Foot, '27) technics. Most of the stains were also combined with the impregnations.

OBSERVATIONS

Fresh material

A study of the living cells of the spleen was not very illuminating, owing to the difficulty experienced in identifying them; many large cells were found to be extraordinarily active, quite as lively as fresh-water amebae, but it could not be determined whether they were all macrophages or whether some of them were not detached endothelium. Cells showing the rod-like morphology of the latter were found, but they were not actively motile. One could make out beaded masses, ar-

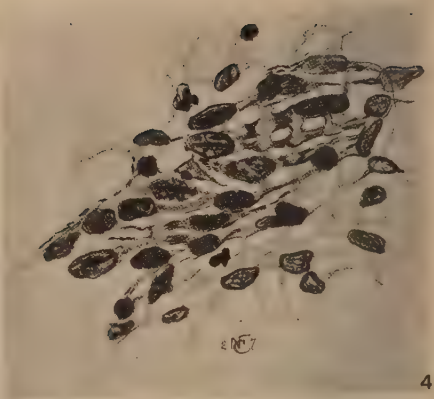
ranged in delicate pairs, along the protoplasmic processes and corresponding to the bands seen in the fixed, stained specimens. These masses were refractile and yellowish. No membrane, nor fragments thereof, could be found in spite of careful searching.

Fixed sections

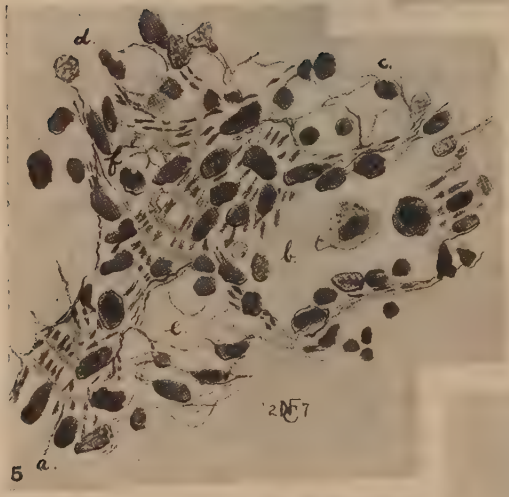
We may now attempt to answer the five questions postulated at the beginning of this paper. As to the first—concerning the morphology of the endothelial cells as units—it can be said that they are, so far as can be judged, elongated and narrow, rod-like rather than fusiform, with a nucleus that bulges into the lumen of the vessel and a cytoplasm that is not homogeneous, but shows definite light and dark areas. From partially teased specimens, obtained from a spleen that was rather vigorously massaged during perfusion, cells were isolated and identified as coming from the venous endothelium. They were also observed in scrapings of fresh spleen, in smears. Their appearance is shown in figure 3. There is no adhering membrane, nor shreds thereof, and they are quite innocent of intercellular bridges or processes that could be considered as the stumps of avulsed bridges.

As to their arrangement in the walls of the venous sinuses and veins, they run lengthwise in both, although they may have a somewhat diagonal course in the former (fig. 4); they lie parallel with one another in all cases, excepting where there is a side branch, when their arrangement becomes complicated, more or less radial, and the bands are thinned out into a delicate network, as shown at *f* in figure 5. Their general arrangement is shown in all the drawings. There is absolutely no indication that the structure of the walls of the venous sinuses differs materially from that of the veins; this is readily demonstrable in well-preserved specimens. There is somewhat more space between the sinus cells, and they converge to become more closely packed where the lumen narrows to become that of a radicle. Any difference of arrangement in the sinus and vein, then, is merely one of adaptation to changes in the caliber of the vessel.

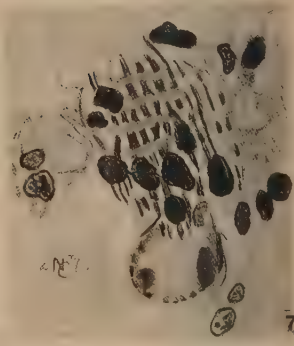
3



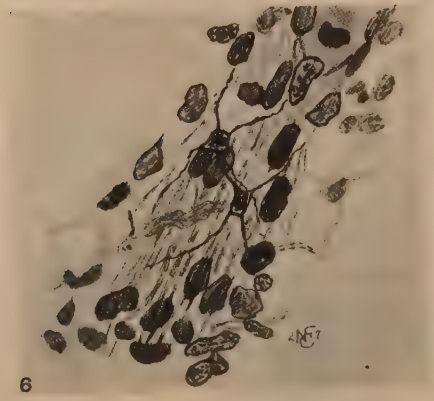
4



5



7



6

8

As to the delicate, structureless protoplasmic membrane that is supposed by some to lie between the endothelium and the reticular framework—every effort was made to demonstrate this, without any success. In eosin-stained sections there was sometimes an indication of membranous structure, particularly at the point of branching of a sinus or vein, but this was merely due to the stain and the rather membranous character of the endothelium at these points. No membrane was seen in fresh teased specimens, none was found in fixed teased tissue, and none could be demonstrated by any method (and many were used) in fixed sections. If there be such a membrane, it must require extremely specialized technic for its demonstration.

Schridde's delicate protoplasmic network, described as lying just outside the endothelium, can be demonstrated by the methods employed in this investigation; it is composed of the delicate, filamentous processes of small, spider-like cells that lie just without the endothelium and extend over its surface. These are pictured in figure 6 and are analogous to the adventitial cells in ordinary capillaries or, possibly, to Rouget cells (Clark and Clark, '25). They do not seem to be uniformly distributed, for one must search before finding them, and they are not very closely arranged in any given area.

Fig. 3 Drawing of partially teased spleen, after perfusion, showing four isolated endothelial cells from the walls of a venous sinus. Note the bands, the marginal thickenings, and the absence of any indication of avulsed intercellular bridges. Phosphotungstic acid hematoxylin.

Fig. 4 Drawing of the floor of a venous sinus, showing the diagonal arrangement of its endothelial elements; same stain.

Fig. 5 Confluence of several venous sinuses—*a*, *b*, and *d*. *C* represents the pulp just below one of these, and *e* is also pulp reticulum. At *f* the bands are rearranged to form a plexus. Same stain.

Fig. 6 Drawing of an intralobular vein, to show the adventitial cells as they lie on the outer aspect of its wall. Same stain.

Fig. 7 Drawing of a venous radicle, to show 'hoops' as they appear in plain phosphotungstic acid hematoxylin stain.

Fig. 8 Drawing of a sinus wall in a section stained with phosphotungstic acid hematoxylin combined with silver-ammonium carbonate impregnation. The black reticulin fibrils do not invariably traverse the 'hoops.'

9

10

11

12

Endothelial striations

These are of prime importance in this paper, as they have been described only by Szymonowicz, so far as can be ascertained. They are readily demonstrable in phosphotungstic acid hematoxylin preparations and they were indicated in Mollier's drawings. The endothelial cells show areas staining blue with phosphotungstic acid hematoxylin and brown with silver tannate, which alternate with bands that stain yellowish with the former and do not become impregnated with the latter. The light areas underlie the reticular 'hoops,' or annular fibers, that surround the vessels and, if phosphotungstic acid hematoxylin be used alone, they appear to be connected by intercellular bridges of the same color (figs. 2 and 7). This is also true of eosin-stained sections. If, however, a silver impregnation be superimposed upon the other stains, it will be seen that the reticulum courses over these bridges, as shown in figures 8 and 9. As some bridges show no reticulum, moreover, it is possible that we are dealing with two elements; a rather broad, pale band of protoplasmic material overlaid in places by a bundle of reticulin fibrils, beyond which the pale band projects on either side. This is poorly defined and would easily pass for a pale area in the endothelium, constituting an intercellular bridge, were it not for the fact that it cannot be demonstrated in teased specimens. If silver-tannate impregnation be employed, there is no sign of the broader band, the whole 'hoop' is impregnated deep brown or black with the silver (fig. 10).

When seen from above, looking down upon the outside of a venous wall, the dark areas appear to be composed of two

Fig. 9 High dry photomicrograph (× 530) to confirm figure 8.

Fig. 10 Drawing of a venous radicle in a preparation impregnated with silver tannate, which has caught reticulin fibrils over all the 'hoops.'

Fig. 11 Transverse and oblique sections of venous sinuses drawn to demonstrate the superficial situation of the endothelial bands or thickenings. The small scale drawn in at the top of the figure will give the magnification for all the drawings.

Fig. 12 Diagrammatic representation of the probable architecture of a venous channel in the spleen. The arrangement in the sinuses may be oblique or spiral, rather than rigidly vertical as in this drawing.

rather darker and longer stripes connected at their middle by a paler middle section; they are somewhat like elongated H's, with thick, poorly defined cross-bars. Seen from the side in longitudinal sections, or teased cells that lie upon their side, or viewed in transverse sections (fig. 11), they appear to occupy a portion of the outer surface, rather than to lie within the cytoplasm; to exist as local thickenings of the ectoplasm rather than darker areas in the protoplasm of the cell body. In isolated cells they often appear twisted and seem to spiral their way over the surface, which is probably due to the fact that these cells are, indeed, twisted and more or less distorted by manipulation. This is seen in figure 3. It has been stated that the bands are separated by lighter intercalations that underlie the annular fibers; this might lead one to infer that these exerted some pressure upon the underlying cytoplasm, forcing the basophilic material into the spaces between the rings, but these areas remain pale after the cells have become detached from the rings.

The import of these bands is not clear: they might represent contractile organs, but we have no evidence that they are contractile, and they are not as distinct as the striae in skeletal muscle; they may represent local thickenings of the cell membrane, or they may be a manifestation of alternating areas of rarer and denser cytoplasm near the cell surface. The second hypothesis seems the most probable, but we cannot explain their function. They are too superficial and dif fuse to be considered mitochondria, they appear too constantly to be artifacts. Why they are present between the supporting rings and are absent just beneath and a short distance to either side of them remains a question.

SUMMARY

To sum up these observations and reconstruct theoretically the typical venous channel in the spleen, it may be stated that it consists of the following elements, as ascertained by the methods enumerated above. The channel wall is composed of rod-like, elongated cells that lie parallel with its long axis

and with one another, separated by narrow chinks, or fenestra. These cells are held in their places by two supporting systems: an inner, delicate network composed of filamentous processes given off by small, quadrate, adventitial cells that are closely applied to the outer aspect of the endothelium; an outer structure is made up of rather broad, ribbon-like bands of protoplasm that stains yellowish with phosphotungstic acid hematoxylin and are usually overlaid by annular and somewhat anastomotic fibrils of reticulin, which may not be demonstrable in every case. No intercellular bridges could be demonstrated between the endothelial cells and no membrane was found to surround them.

The endothelial cells are characterized by their shape and by the possession of superficial thickenings that suggest muscle striae, but lack the definite morphology of these. Occasionally, mononuclear cells are seen lying in an almost unbroken circle at the periphery of the vessel, just within the true endothelium, and when they are numerous, they have the appearance of a more or less swollen endothelial lining. These cells, however, are easily washed out by perfusion and probably represent laggards in the venous circulation that lie along the endothelium and enter or leave the vessels through the fenestra between the endothelial 'staves.' Figure 12 will graphically represent the conception of the structure of a venous channel as set forth in this paper; it has been drawn somewhat diagrammatically in the manner of Mollier's familiar illustration.

LITERATURE CITED

Bailey 1926 Bailey's text-book of histology, O. S. Strong and A. Elwyn, 7th ed., p. 253. New York.

Clark, E. R., and Clark, E. L. 1925 The development of adventitial (Rouget) cells in the blood capillaries of amphibian larvae. Am. Jour. Anat., vol. 35, p. 239.

Foot, N. C. 1927 A silver tannate technic for paraffin sections from the central nervous system. Arch. Path., vol. 4, p. 36.

——— 1927 The reticulum of the human spleen. Anat. Rec., vol. 36, p. 79.

MacCallum, W. G. 1921 A text-book of pathology, 2nd ed., p. 802. Philadelphia and London.

MALLORY, F. B. 1923 Principles of pathologic histology, p. 610. Philadelphia and London.

MOLLIER, S. 1910–1911 Ueber den Bau der capillaren Milzvenen (Milzsinus). Arch. f. mikr. Anat., Bd. 76, S. 609.

ROBINSON, W. L. 1926 The vascular mechanism of the spleen. Am. Jour. Path., vol. 2, p. 341.

SCHRIDDE, H. 1923 In Aschoff's Pathologische Anatomie, 6te Aufl., Bd. 2, S. 140. Jena.

SZYMONOWICZ, L. 1924 Lehrbuch der Histologie und der mikroskopischen Anatomie, 5te Aufl., S. 169. Leipzig.

STÖHR, P. 1924 Lehrbuch der Histologie und der mikroskopischen Anatomie des Menschen, u.s.w., 20ste Aufl., neubearb. von Dr. W. Möllendorff, S. 176. Jena.

THIEL, G. A., AND DOWNEY, H. 1921 The development of the mammalian spleen with special reference to its hematopoietic activity. Am. Jour. Anat., vol. 28, p. 279.

NOTES ON THE BLOOD VESSELS OF THE BRAIN IN A HOLOCEPHALIAN—HYDROLAGUS (CHIMAERA) COLLIEI

E. HORNE CRAIGIE

Department of Biology, University of Toronto, and the Pacific Biological Station, Nanaimo, British Columbia

TWO FIGURES

Several specimens of the ratfish, Hydrolagus colliei, Lay and Bennett, were procured by the writer during a recent visit to the Pacific Biological Station, Nanaimo, British Columbia, and three of these were injected with carmine gelatin from the heart in an attempt to fill the blood vessels of the brain. Two of these injections were successful, and the brains were fixed and cut in transverse sections 20 μ thick. A quantitative study of the capillaries in these was the subject of a separate report (Craigie, '27). In the third case only the larger vessels on the surface of the brain were filled with the mass, so the whole head was preserved for gross dissection. Unfortunately, it was not suspected at the time that the blood vessels of the brain of Chimaera had not been described, and only after the successfully injected material had been embedded did a search fail to reveal such a description.

Although the material is too limited as a basis for general conclusions, the holocephalian type is such an interesting one that a brief description of the superficial arteries of the brain and of the distribution of the vessels within its substance is believed to be worthy of publication.

THE ARTERIES OF THE BRAIN

The somewhat peculiar relations of the carotid arteries in Hydrolagus have been accurately described and figured

by Allis ('12), and the writer has been able to confirm his description by a dissection of the partially injected head mentioned above and of two uninjected specimens.

The brain does not receive any blood through the internal carotid arteries, the only source of supply, other than that from behind through the spinal and basilar arteries, being the so-called anterior carotid arteries (Parker, '86; Allis, '12). The latter are the equivalent of the combined afferent and efferent vessels of the spiracular pseudobranch of elasmobranchs, these forming one continuous vessel in the absence of the spiracle, while the distal portion, as Allis states, is probably the anterior part of the internal carotid which has become disconnected from its original stem during development.

The anterior carotid artery at the point where it emerges into the cranial cavity, just level with the optic chiasma, and still close to the cranial wall, divides into anterior and posterior cerebral arteries and sends a small branch posteromedially straight across the intervening space to the ventral surface of the inferior lobes and the vascular sac. A secondary branch from this vessel crosses to the optic nerve and accompanies it toward the periphery.

The posterior cerebral artery, which is considerably the larger of the two, passes posteriorly and somewhat medially to meet the lateral surface of the brain immediately dorsal to the inferior lobe of the hypothalamus. It curves posteromedially round the latter region, and immediately behind it, between the roots of the oculomotor nerves and dorsal to the vascular sac, unites with its fellow of the opposite side to form the basilar artery. The two posterior cerebral arteries meet at approximately a right angle. Before reaching the surface of the brain, the posterior cerebral gives off a small artery which ramifies over the side of the diencephalon and the front of the inferior lobes, and just behind it another somewhat larger branch which runs dorsally on to the upper surface of the diencephalon and then turns backward on to the optic lobe. Immediately upon reaching the brain the

posterior cerebral artery gives rise to a branch passing over the posterior surface of the inferior lobes to the dorsal aspect of the vascular sac. Just at the point of union of the two

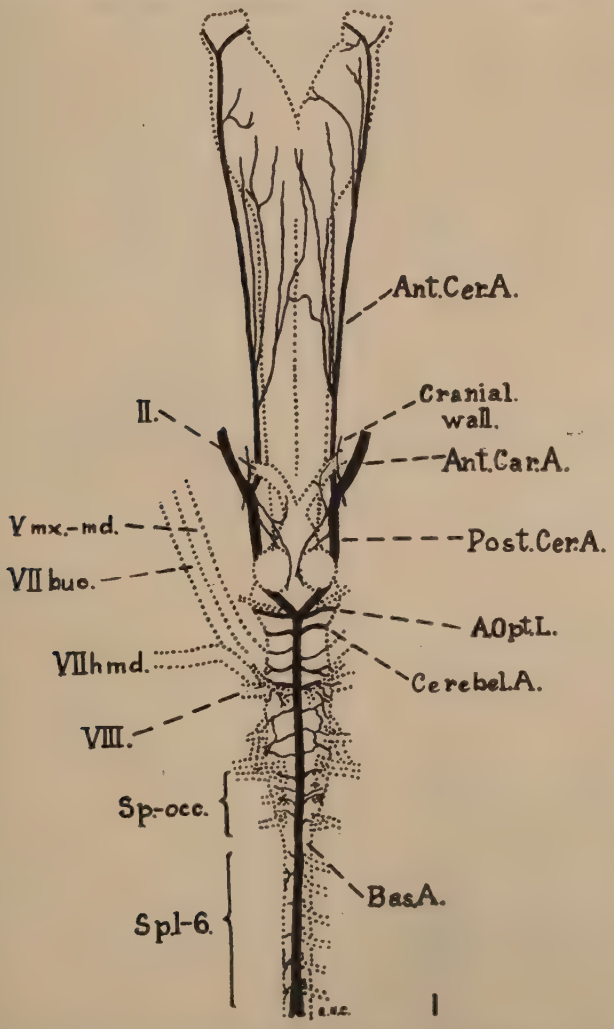


Fig. 1. Ventral view of the arteries of the brain of the ratfish (Hydrolagus colliei).

posterior cerebral arteries, a vessel of considerable size, the artery of the optic lobe, passes out at either side to the posterior portion of the lateral surface of the mesencephalon and branches dorsally and anteriorly over the optic lobe, also anastomosing backward with the cerebellar artery.

The basilar artery extends straight back to become continuous with the anterior spinal artery without any very marked change of caliber. It gives off along its course about a dozen pairs of branches which pass laterally and dorsally to ramify over the whole surface of the hindbrain. The largest pair arises just behind the origin of the arteries of the optic lobes. These are the cerebellar arteries, which supply the cerebellum and send branches (myelometencephalic arteries

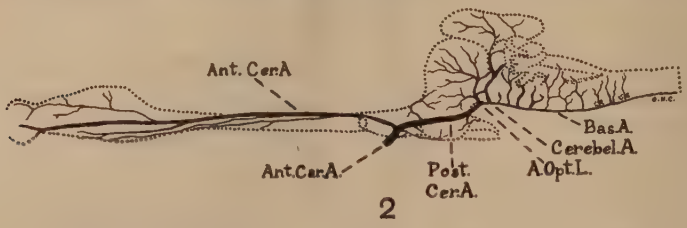


Fig. 2 Lateral view of the arteries of the brain of the ratfish.

—Sterzi) back into the anterior part of the acusticolateral lobe. The cerebellar artery is connected by two anastomotic branches with the artery of the optic lobe in front of it (fig. 2), the more distal of these being so large that the vessel distributing to the anterior portion of the corpus cerebelli might be considered as coming from the former rather than from the latter vessel. The succeeding vessels supply the corresponding parts of the medulla oblongata, including the acusticolateral lobes and the nerve roots, the branches to the trigeminus and to the acusticus being noticeably large.

Farther back, similar but smaller, segmentally arranged branches spread over the surface of the spinal cord.

There appear to be minute vessels accompanying at least some of the occipital and anterior spinal roots, but they are very small and do not show clearly in the specimen examined.

Just at the level of the sixth spinal nerves, where the specimen studied is cut off, the anterior spinal artery divides into two, presumably to form a loop, after expanding a little in the two preceding segments.

The anterior cerebral arteries are of smaller caliber than the posterior ones. They turn anteriorly over the optic nerve, but do not appear to supply a branch to the latter structure. They then run straight forward along the side of the narrow, elongate cranial cavity, giving off long branches which pass anteromedially to the ventral surface of the extended posterior portion of the telencephalon, where they form a system of superficial rami, and pass forward on to the ventral surface of the hemispheres more anteriorly. At least one distinct anastomotic branch connecting rami from the two sides was observed (fig. 1), this being situated somewhat less than half-way forward between the optic chiasma and the anterior end of the brain. Thus a complete circle of Willis is present, in this specimen at least of Hydrolagus, which differs from other fishes in this respect, though the anterior anastomotic vessel is very small. Upon reaching the larger anterior parts of the telencephalon, the anterior cerebral artery sends out small branches over it. At the base of the olfactory bulb it divides into medial and lateral rami, of which the latter penetrates the olfactory sac. The medial ramus crosses the ventral surface of the olfactory bulb obliquely to its anteromedial aspect and then runs dorsally up the anterior wall of the cranial cavity and also enters the olfactory sac.

These arteries appear to correspond more closely with those of Scyllium than with those of Acanthias, according to the description of Sterzi ('09). The main vessel passing forward apparently represents the combined anterior cerebral and olfactory arteries of the latter. Its relations correspond rather with those of the lateral cerebral arteries of Sterzi's description of Scyllium, but it is the medial artery in that type which supplies the olfactory sac. The more medial and posterior of the long branches may represent the medial cerebral artery, the others being branches of distribution of

the lateral vessel. All are, of course, greatly extended anteroposteriorly as a result of the elongation of the telencephalon medium characteristic of the holocephalian brain.

VESSELS IN THE BRAIN SUBSTANCE

Thin sections of material from which the meninges were largely stripped off before embedding do not show satisfactorily the precise arrangement and relations of the vessels, but the following observations were made.

The arterioles and venules penetrating the substance of the spinal cord and brain (which vessels are indistinguishable from each other in this material) are very small, and mostly have walls little or not at all thicker than those of the capillaries. This corresponds to the description of the vessels of Acanthias given by Sterzi, as does also the arrangement of the vessels in the cord, where they penetrate the surface in greatest numbers opposite the dorsal and ventral gray columns, into which they extend. Central arteries arising directly from the anterior spinal artery and running dorsally in the median septum are very few and small. Occasional small vessels accompanying the nerve roots into the substance of the cord were also observed.

In the medulla oblongata the arterioles and venules are mostly arranged in a somewhat radiate manner, and many are larger and thicker-walled than most of those observed in the cord, many (venules) having diameters of 20 μ to 35 μ as measured on the slide. They break up into the capillary network which fills the reticular formation and the gray centers, the larger arterioles mostly extending in about as far as the central gray matter apparently. The radiate arrangement of even the smaller vessels is particularly marked in the cerebellar crest. Subependymal venules and veins are arranged much as described by Sterzi for Acanthias.

The cortex of the cerebellum has a rich capillary net, but the arterioles and venules penetrating its surface are not specially numerous and are mostly small in caliber. If they have any

special arrangement such as is described for the dogfish, the sections are too thin to show it. The vessels in the cerebellar columns (granular layer), however, appear to spread through these structures in a fan-shaped manner from the sagittal sulcus, as in Acanthias.

The arrangement in the mesencephalon and diencephalon presents nothing of note. The vessels, as elsewhere, penetrate the surface more or less perpendicularly at points all over its extent and break up quickly into a capillary net.

In the elongated telencephalon medium, since this region is made up of longitudinal tracts, i.e., of white matter, the blood vessels are not numerous. Small vessels penetrate the outer surface here and there and mostly pass directly inward. In the zone between the parallel fiber tracts some at least of these vessels turn or branch at right angles, and it appears possible that they may form here a network with very large meshes in the one plane. In thin sections, of course, it is impossible to tell whether they actually connect in this way. Internally, they again appear running perpendicular to the surface and some at least enter subependymal veins. The greatly expanded membranous roof has a venous plexus, as would be expected, and this extends down into a median fold projecting into the ventricle.

The corpora striata and parts superficial to them receive numerous arterioles of capillary size, which evidently must arise from ramifications of the long branches of the anterior cerebral artery. Vessels of considerable size (up to 40 μ or more), presumably venules, appear running in various directions through the tissue. It would seem that the venules empty chiefly into the meningeal network.

Farther forward, all parts of the hemisphere, both pallial and subpallial, are seen similarly to be dependent upon small arterioles penetrating at points all over the peripheral surface. The general arrangement of the vessels in the hemisphere is distinctly radiate. The capillaries empty into venules similar to those noted in the posterior part of the hemisphere, many of which appear to unite into somewhat

larger vessels in a subependymal position whence they may be observed extending right out to the peripheral venous plexus in the meninx.

SUMMARY

1. As is already well known, the sole source of blood supply for the brain of Hydrolagus, other than that from behind, is the anterior carotid artery.

2. This divides into anterior and posterior cerebral arteries, of which the ramification and distribution are described.

3. The relations and distribution of the posterior cerebral and basilar arteries resemble those in selachians.

4. The anterior cerebral and its more posterior branches are greatly elongated, in correspondence with the elongation of the telencephalon medium. The main vessel, after giving off branches to the hemisphere, divides into two terminal rami which penetrate the olfactory capsule. There is a small transverse anastomosis between the two sides.

5. The distribution of the vessels within the brain substance is also described. In general, it resembles the conditions in selachians, as described by Sterzi.

LITERATURE CITED

Allis, E. P. 1912 The branchial, pseudobranchial, and carotid arteries in Chimaera (Hydrolagus) colliei. Anat. Anz., Bd. 42, S. 10–18.

Craigie, E. H. 1927 Notes on the vascular supply in the brain of a Holocephalian-Hydrolagus (Chimaera) colliei. Jour. Comp. Neur., vol. 43, pp. 193–200.

Dean, B. 1906 Chimaeroid fishes and their development. Carnegie Inst. of Washington, publ. 32, pp. 1–195.

Hofmann, M. 1900 Zur vergleichenden Anatomie der Gehirn- und Rückenmarksarterien der Vertebraten. Zeits. f. Morph. u. Anthrop., Bd. 2, S. 247–322.

Parker, T. J. 1886 On the blood-vessels of Mustelus antarcticus: a contribution to the morphology of the vascular system in the Vertebrata. Phil. Trans. Roy. Soc., vol. 177, pp. 685–732.

Sterzi, G. 1909 Il sistema nervoso centrale dei vertebrati, vol. 2, libro 1: Selaci.

de Vriese, B. 1905 Sur la signification morphologique des artères cérébrales. Arch. de Biol., T. 21, pp. 357–457.

RELIABLE METHODS FOR THE FIXATION AND STAINING OF NISSL SUBSTANCE

P. ARTHUR DELANEY
The Anatomical Laboratory of the University of Chicago

There have been many and varied contributions to the literature bearing upon staining methods for Nissl substance and related nervous histology. When one refers to well-recognized texts on microscopic anatomy methods and to other scientific publications, there are found many conflicting opinions as to what technique of fixation and staining is suitable for temporary or relatively permanent preparations of this substance. This article aims to report, first, a method whereby Nissl substance stains rapidly and brilliantly and which at the same time differentiates much related histology, and, secondly, some very elucidating results that were obtained through experimentation with different fixatives, aqueous and alcoholic, and through modifying the reaction of the dye diluent used.

SOURCE OF MATERIAL

Although, to my knowledge, the staining method to be described has given very excellent results with tissues from the central nervous system of man, monkey, and dog, the material used in securing the results cited was from the cat, and included individual segments of the spinal cord with related posterior nerve-root ganglia, at different cord levels from the cervical to the sacral region. One series of spinal cord and ganglia was from a fullgrown animal, while two other series were obtained from two cats of the same litter, about ten days old.

FIXATIVES USED

Since early in the investigation it was found that the aqueous fixative, formalin Zenker, provided material that could be stained in a most satisfactory manner, it was decided to select a group of different fixatives, some aqueous, others alcoholic, that their relative values might be ascertained. Many of the more important contentions of investigators were kept in mind, and, adopting a uniform time period of twenty-four hours at room temperature for all fixatives, the following were used:

Aqueous

1. Formalin Zenker:	
Zenker's fluid,	90.0 cc.
Formalin,[1]	10.0 cc.
2. Acetic Zenker:	
Zenker's fluid,	95.0 cc.
Glacial acetic acid,	5.0 cc.
3. Bouin's fluid:	
Picric acid, saturated aqueous solution,	75.0 cc.
Formalin,	25.0 cc.
Glacial acetic acid,	5.0 cc.
4. 10 per cent neutral formalin:	
Formalin,	10.0 cc.
Distilled water,	90.0 cc.
5. Zenker's fluid:	
Potassium dichromate,	2.5 grams
Mercuric chloride,	5.0 grams
Distilled water,	100.0 cc.
6. Bensley's 90-10-3:	
2.5 per cent potassium dichromate,	90.0 cc.
Formalin,	10.0 cc.
Glacial acetic acid,	3.0 cc.
7. Regaud's fluid:	
A. 3 per cent potassium dichromate,	80.0 cc.
Formalin,	20.0 cc.
B. 3 per cent potassium dichromate.	

Material was placed in A for three to four days, changing the fixative daily, and in B for six days, the fixative being renewed every other day.

Alcoholic

1. Carnoy's fluid:	
Absolute alcohol,	60.0 cc.
Chloroform,	30.0 cc.
Glacial acetic acid,	10.0 cc.

[1] In this fixative and in others where formalin was used, it was of concentrated strength and neutralized with magnesium carbonate.

2. Absolute alcohol, 90.0 cc.
Formalin, 10.0 cc.

3. Alcoholic chrome sublimate, consisting of equal parts of 95 per cent alcohol saturated with mercuric chloride, and 3.5 per cent aqueous potassium dichromate.

4. 70 per cent alcohol.

5. 95 per cent alcohol.

EMBEDDING AND SECTIONING METHODS

Conventional methods of dehydration and of paraffin and celloidin embedding were used. In the paraffin-embedding technique tissues were cleared in cedar oil. For celloidin embedding, Dupont parlodion was used either exclusively or else in about equal quantity with a special celloidin prepared by Professor Bensley. In the paraffin series sections were cut 7 μ to 8 μ thick and mounted on slides with Mayer's glycerin-albumin fixative by the water method. Celloidin sections were cut about 10 μ thick and stained as free sections.

STAINING TECHNIQUE

Different staining combinations will be referred to in the discussion. There will now be described the method that has proved generally to be best. In the case of paraffin sections, these are freed of their embedding agent and brought to distilled water. Better arranged on a staining rack, they are covered with a filtered solution of Loeffler's alkaline methylene blue made up as follows:

Grübler's methylene blue rectified, saturated in absolute alcohol, 30 cc.
Potassium hydroxide 1–10,000 in distilled water, 100 cc.

Heat from an alcohol lamp is then applied until steam arises. The author generally heats until the alcohol content of the staining solution ignites, and then discontinuing heating allows the stain to cool, when it is washed off with distilled water. Where preferred, the stain may be allowed to act without heating for twenty to thirty minutes. The stained section is then differentiated in the following:

Alcohol soluble eosin, saturated in 95 per cent alcohol.

This eosin stain may be diluted with 95 per cent alcohol to any strength, if a slower decolorizing and counterstaining action is desired or necessary. When the material has been fixed in alcoholic fixatives, the methylene blue stains intensely and diffusely, and it is recommended that the major part of the decolorization be done independently in 70 to 80 per cent alcohol, else the red counterstaining may be too intense; with aqueous fixatives the methylene blue penetrates well, but to a lesser degree, and often it is necessary to dilute the saturated alcoholic eosin with 95 per cent alcohol from five to ten times. In this way one can control decolorization and counterstaining to a very exact color contrast. Aqueous eosin of varying strength may be used, but here the action of differentiation is very slow and is hardly to be recommended where material has been treated with alcoholic fixatives.

When celloidin sections are to be stained, the same principles of staining are adhered to, except that now the sections are placed in a small glass dish or beaker, where they are covered with the methylene-blue stain and heated gently until steam makes its first appearance; heating is now discontinued, the solution allowed to cool, and the same degree of heating is repeated. This precaution in heating preserves the celloidin. From 5 to 10 per cent of chloroform must be added to the alcoholic eosin stain, and to the higher alcohols and alcohol-xylol mixture, that the celloidin may not be dissolved.

It is recommended that sections from differently fixed material be stained separately, and that the rapidity of differentiation by the alcoholic eosin be first ascertained by experimenting with a few sections, having frequent recourse to the microscope, and taking into consideration the fact that some of the methylene-blue color will be extracted by the subsequent treatment with the higher alcohols, before mounting. It has been found advantageous to carry the sections from the alcoholic eosin quickly through two changes of absolute alcohol, one change of equal parts of absolute alcohol and xylol, and then through two changes of xylol, finally bringing

the sections to some fresh xylol or toluol. It appears that sections that have not been entirely freed of alcohol fade in a comparatively short time.

MOUNTING OF STAINED SECTIONS

No experimentation has been done to determine if different balsams affect the staying qualities of the stain. In this work the sections were mounted in commercial balsam that had been neutralized by a petroleum-ether method devised by Professor Bensley.

DISCUSSION OF EXPERIMENTAL METHODS

The matter of fixatives in their relation to staining possibilities will be considered first. Under previous reference to them in this article, those used in this work have been listed under aqueous and alcoholic groups, and in the order of superiority in which they are regarded in each group.

Of the aqueous fixatives used, formalin Zenker, proves to be a most excellent fixative. It penetrates well and evenly the spinal cord and posterior nerve-root ganglia. After staining, the Nissl substance in the large motor cells of the anterior horn appears as large discrete bodies colored blue to purple, while in the smaller nerve cells of the cord it has more of a granular consistency, but these granules are large and discrete. In the posterior nerve-root ganglia, where chromatolysis is frequent and cytology varies, the picture of the Nissl substance is excellent and instructive. In addition, nuclei, nucleoli, nerve fibers, epithelium of the central canal, the cord coverings, and other related structures appear distinctly stained by the methylene blue or eosin.

Acetic Zenker is less penetrating than formalin Zenker. This manifests itself through the somewhat poorer picture that the motor cells of the anterior horn display, but where the posterior nerve-root ganglion is small, as in the cat about ten days old, the array of cells with their Nissl-substance content gives a picture that no fixative used, aqueous or alcoholic, has excelled.

Fixation with Bouin's fluid, with 10 per cent neutral formalin, or with Zenker's fluid without formalin or acetic acid, has given satisfactorily stained pictures that show too insignificant individual superiority to merit discussion. Although to be considered worthy of trial, none of them is considered as ranking in efficiency with either formalin Zenker or acetic Zenker.

The Bensley fixative of potassium dichromate, neutral formalin, and glacial acetic acid—a formula that gave Professor Bensley some satisfactory results in his study of the hypophysis—and Regaud's fluid as used by Doctor Ma in his study of mitochondria and secretion granules, both proved to be of no value. The Nissl bodies did not take any of the methylene-blue stain. With Regaud's fixative, the myelin of the nerve fibers presented a very peculiar appearance.

Consideration of the results described as obtained with aqueous fixatives necessarily point out that, in the matter of fixation of Nissl substance, alcohol is not an absolute essential, neither is the use of the bichromates to be avoided, as has been urged where the Nissl method of staining is to be used.

Of the five alcoholic fixatives used, none of them permitted results of staining to be obtained that equaled those seen with formalin-Zenker or acetic-Zenker fixation. All of them gave results, however, and it must be acknowledged that Carnoy's fluid is very dependable and satisfactory, not inducing as much tissue shrinking as the other alcoholic fixatives. The absolute alcohol-formalin fixative listed under 2 of the alcoholic group is one that has been used with success by Mr. J. O'Leary, a student in this department, in the study of glycogen. Generally, material fixed in solutions containing alcohol stain very deeply and diffusely with the methylene blue, and the process of differentiation does not have to be hurried. Nevertheless, alcoholic fixatives produce marked tissue shrinkage that is to be avoided whenever possible. The different alcoholic fixatives used have been appraised largely with this last unsatisfactory feature primarily considered.

In the matter of embedding, the contention that "paraffin should be used only when unavoidably necessary or for special purposes," and that "pieces to be embedded in celloidin are not to be passed through alcohol-ether, but directly from absolute alcohol into thin celloidin," being considered, the spinal cords with ganglia from the two cats about ten days old were cut and corresponding segments fixed in the twelve different fixatives cited. One series was embedded in paraffin, the other in celloidin, as per methods previously described. In the celloidin process of embedding the pieces of tissue were placed in equal parts of absolute alcohol and ether for twenty-four hours, and had further action of the alcohol-ether through a series of dilute celloidins of 1, 2, 3, 4, 6, and 8 per cent and on to the thick celloidin of 18 per cent concentration. That the beautiful results described as obtainable with material fixed in formalin Zenker or in acetic Zenker apply to paraffin- and celloidin-embedded tissues alike necessarily characterize these admonitions as very unimportant, and to be disregarded at least when the staining procedure that this article has included as the important part of its contribution is used. As most excellent results have been obtained both after paraffin and celloidin embedding, the matter of a choice between the two had better be a personal one, suited to the more urgent needs of the user.

In the matter of staining the Nissl substance, the only dye used has been methylene blue. Early in the investigation, however, contrast studies were made by using four different dyes in the making of Loeffler's alkaline methylene blue. These dyes are here listed: 1) Grübler's methylene blue, rectified. 2) Grübler's methylene blue, bacterial. 3) Methylene blue prepared by the Lindsay Light Co., Chicago. 4) Parke-Davis medicinal methylene blue.

The Grübler's methylene blue rectified generally was found to be superior, and especially so when used with material fixed in aqueous fixatives. Sections from material treated with alcoholic fixatives often gave better pictures with the dyes prepared by the Lindsay Light Co. and by the Parke-

Davis Co.; their lesser penetrating power allows the differentiation to be effected more rapidly.

The matter of differentiation by means of alcoholic or aqueous eosin has been fully covered under 'staining technique,' and need not be discussed here. It might be stated that 0.02 per cent aqueous acid fuchsin gave satisfactory differentiation when used with material treated with aqueous fixatives. Though never used where alcoholic fixatives had been employed, it might prove efficient in a slightly stronger solution. This acid dye differentiates very rapidly, and can only be used in a very weak solution.

Further experimenting was done, aiming to determine to what extent changes in reaction of the diluent of the alcoholic methylene blue affected the penetration of the stain. In every case, 30 cc. of a saturated alcoholic solution of Grübler's methylene blue rectified was used with 100 cc. of diluent, or proportionate amounts. The diluents used were as follows:

1. Potassium hydroxide, 1 part in 5,000 parts of distilled water.
2. Potassium hydroxide, 1 part in 7,500 parts of distilled water.
3. Potassium hydroxide, 1 part in 10,000 parts of distilled water.
4. Potassium hydroxide, 1 part in 15,000 parts of distilled water.
5. Potassium hydroxide, 1 part in 20,000 parts of distilled water.
6. Distilled water.
7. Concentrated glacial acetic acid, 1 part in 100,000 parts of distilled water.
8. Concentrated glacial acetic acid, 1 part in 50,000 parts of distilled water.
9. Concentrated glacial acetic acid, 1 part in 1,000 parts of distilled water.

The material used was from two pieces of spinal cord and posterior nerve-root ganglia embedded in paraffin. One piece had been fixed in formalin Zenker, the other in acetic Zenker. Sections from each piece were mounted on the same slide, and thus it was possible to compare further and accurately the relative values of these two aqueous fixatives. Careful study of many slides revealed scarcely any perceptible variation in the Nissl staining where the alcoholic methylene-blue diluent was from 2 to 7 of the foregoing list. The penetration of the methylene blue showed some diminution where it had been diluted with potassium hydroxide of 1 to 5000 or with acetic acid of 1 to 50,000 strength. With the acetic acid

content of 1 to 1000, the methylene blue stained poorly; there was least blue coloring in the posterior nerve-root ganglion of the cord segment fixed in acetic Zenker, and most in the anterior-horn cells of the formalin-Zenker-fixed segment. These results cannot but point out that where Nissl substance has been properly fixed, it may be stained with a good quality of methylene-blue dye, such as that prepared by Grübler, without having to adhere to any strict formula of definite alkalinity.

In conclusion, it may be stated that properly fixed material, stained and mounted as per directions given under specific headings in this article, has displayed a very satisfactory permanency. Many sections so prepared thirty months ago, when the method was first used, are still beautiful, with distinctly blue or purple Nissl substance; a few sections show some slight fading of the eosin counterstain.

PECULIARLY ARRANGED CONNECTIVE TISSUES ENDING IN EPIDERMIS

P. ARTHUR DELANEY

The Anatomical Laboratory of the University of Chicago

TWO FIGURES

Histologists and dermatologists have concerned themselves greatly with the actual and possible relations of contiguous connective tissues and epithelium, especially in the skin. In 1906, Dr. F. Krauss, in his publication[1] on the epidermis-cutis relation in lizards and crocodiles, described and illustrated collagenic fibers ending in epidermis, and stressed the marked infrequency with which elastic fibers entered the basal epithelial cells. In his paper he considered at length the history of the subject and included an extensive bibliography. The present article aims to describe briefly a striking example of collagenic and elastic fibers finding their way into, or out of, the basal cells of the epidermis in the Trionyx muticus, a species of soft-shelled turtle.

The material studied included the whole thickness of the carapace from its posterolateral margin to the lateral body wall. The pieces removed immediately after killing the animal were fixed in formalin Zenker, and the whole animal was placed in the same fluid to provide material for orientation. Some of the pieces were embedded in paraffin, others in celloidin. Sections 8 μ to 10 μ thick were cut in the transverse plane and in the longitudinal plane and stained by Mallory's triple connective-tissue stain, or Mallory's phosphotungstic acid haematoxylin, or by a method recom-

[1] Der Zusammenhang zwischen Epidermis und Cutis bei Sauriern und Krokodilen. Dr. med. F. Krauss., Archiv. f. mikrosk. Anat., Bd. 67, 1906, S. 319–363.

mended to me by Professor Bensley consisting of staining in Weigert's resorcin fuchsin, followed by haematoxylin and thiazin red.

The characteristic flexibility of the carapace of the Trionyx muticus is due, in large part, to two strata of dense white fibrous connective tissue on the dorsal and ventral sides, separated from each other by a median stratum composed of loose connective tissue, nerve fibers, blood vessels, chromatophores, fat, and a ground-substance of fine reticulated nature. These two strata are continuous at the margin of the carapace. On both sides, between the stratum of dense white fibrous connective tissue and the epidermis, is a subepithelial layer of loose connective tissue. Traversing this layer in a direction more or less perpendicular to the epidermis are strands of connective tissue, of more or less uniform thickness, which extend to the epidermis and become lost in the basal layer. The structure of the lateral carapace, as illustrated in figure 1, may be epitomized as follows: 1) Dorsal epidermis. 2) Dorsal layer of loose irregularly arranged connective-tissue. 3) Dorsal stratum of dense connective tissue. 4) Median stratum of loose connective tissue. 5) Ventral duplicate of stratum 3. 6) Ventral layer of connective tissue corresponding to stratum 2. 7) Ventral epidermis.

The two strata of dense connective tissue referred to above under 3 and 5 present peculiar architectural designs of duplicate pattern. Marginal pieces of tissue extending for about 2 cm. toward the vertebral column show these two strata to be of equal thickness. At the margin where dorsal and ventral surfaces meet, they unite and have a combined thickness of about 0.15 mm. Receding medially from this point, both layers display a progressive increase in thickness, and 2 cm. from the margin have an individual measurement of 1.40 mm. This rapid change in thickness is effected through an increase in the number and size of definitely separated bundles or fasciculi of dense, regularly arranged, white fibrous connective tissue. At the margin, where the strata meet and are about 0.15 mm. thick, there are two bundles, a lateral bundle

with fibers cut across, whose direction is anteroposterior, and an inner bundle displaying fibers cut along their length, as they extend lateromedially. At the inner margin, approximately 2 cm. from the edge of the carapace, the 1.40-mm. thickness is made up of eleven bundles, six extending anteroposteriorly and five lateromedially, arranged in regular alternating fashion. A third connective-tissue layer completes a very striking lattice-work effect. Its many continuous, flattened plates of connective tissue traverse the varying thickness of the strata, at intervals controlled by the size of the alternating bundles or fasciculi described. As the plates lie between the anteroposterior bundles cut across, they appear as thin sheets separating contiguous fasciculi; but in relation to the adjacent fasciculi cut lengthwise, these plates appear spread like a fan between the fasciculi. Leaving these strata, the connective-tissue fibers of these plates appear as strands that extend into the median stratum of loose connective tissue, branching freely. Peripherally, these strands contribute the interesting connective-tissue bundles that traverse the subepithelial layer of connective tissue and reach the epidermis. Thus the fibers in question traverse the entire thickness of the lateral margin of the carapace from the dorsal to the ventral epidermis and effect direct union with both.

The subepithelial layers of connective tissue, in addition to the traversing connective-tissue strands, are made up of blood vessels, nerve fibers, fibroblasts, crystalloid eosinophiles, and other wandering cells, in a supporting framework of fine reticular tissue similar to that already mentioned as present in the median stratum. In this layer, on the dorsal side only, there are many chromatophores varying greatly in morphology. Generally there are present near the epidermis two or three narrow, tendinous layers alternating in direction similarly to those of the dense connective-tissue strata. Through these comparatively loose strata, the traversing connective-tissue strands follow a tortuous course, infrequently coalescing, and seldom branching except as they approach the epidermis.

Figure 1

Figure 2 illustrates the striking distribution of the collagenic fibers as they enter the basal cells of the epidermis. Each fibrous strand as it divides in relation to the base of the epidermis provides many penetrating fibrillae to each basal cell within its range of distribution. Its peripheral fibrillae appear to join the analogous fibrillae of the adjacent fibers, thus forming a distinctly continuous connective-tissue basement membrane. The greater portion of the fibrillae, however, enter the basal epithelial cells at frequent intervals uniformally separated; their outline is very clear in the lower third of the cell, and not infrequently basal cells are seen with fibrillae penetrating them as far as the nucleus which has a constant position in the peripheral end of the cell. In some instances Mallory's triple connective-tissue stain displays basal epithelial cells with red-stained intracellular fibrils resembling tonofibrillae, running along the length of the cell; these project between the blue-stained connective-tissue fibrils and give the cell a very spiny basal contour.

The elastic connective-tissue content is very clearly demonstrated by staining sections with Weigert's resorcin fuchsin, Ehrlich's acid haematoxylin, and thiazin red—a most satisfactory method recommended by Professor Bensley. It is most abundant in the median stratum and in the contiguous collagenic bundles of the adjacent dense strata. Elastic fibers also form part of the thin connective-tissue plates described as part of the compact strata. It is in the subepithelial loose connective-tissue layer that the few elastic fibers forming part of the collagenic-tissue strands are most conspicuous. They stand out as gun-metal blue homogeneous fibers, in the red-stained tortuous, collagenic strand, and can be traced to their entrance into the basal epithelial cell; here their distribution corresponds to that of the collagenic fiber in degree, but not in character. Being devoid of fibrillae, the elastic fiber gives off more or less perpendicular processes that enter the basal cells. Figure 2 illustrates this relation.

Fig. 1 Illustrating the entire thickness of a portion of the carapace of Trionyx muticus. Parts are numbered in the order in which they have been epitomized on page 122.

Fig. 2 A dorsal portion of the carapace of Trionyx muticus, including *1*, *2*, and part of *3*, illustrated in figure 1. The black elastic fibers course through the grayish collagenic fibers and both enter the basal cells of the epidermis.

The epidermis contains two distinct strata: a stratum corneum and a stratum germinativum. There is no stratum lucidum or stratum granulosum. The stratum corneum is a markedly compact layer of indistinctly fibrillar structure without any visible cell outlines. It is slightly thicker on the dorsal than on the ventral surface, and fine brown granules appear irregularly distributed in the dorsal horny layer. The stratum germinativum is made up of from five to ten cell layers, the contour of each cell being distinctly mapped out by spiny processes. The cells composing the layers adjacent to the stratum corneum are squamous with a flattened, vesicular nucleus; those of the basal layer are tall cuboidal to columnar, while the cells forming the intervening layer or layers are low cuboidal with a round or oval nucleus. The thickness of the basal cell layer is very uniform, but its columnar cells are of two distinct types, one wide, the other narrow. The narrower cell invariably displays intracellular fibrillae running along the long axis of the cell and coming to lie in intimate association with the penetrating connective-tissue fibrillae described. The nucleus in both these columnar cells is round or oval, vesicular, with a conspicuous nucleolus, and, as heretofore mentioned, peculiarly located in the outer end of the cell. Although the ventral epidermis constantly has the greater number of cell layers in its stratum germinativum, the dorsal epidermis equals or slightly surpasses it in thickness, because it has a thicker stratum corneum. Among the cells of the stratum germinativum are occasional threads of brown pigment granules.

Sections of the carapace margin representing the anteroposterior axis add nothing to the picture. In them the fibers of the alternating bundles or fasciculi in the strata of dense connective tissue and in the subepithelial layers appear cut in a reverse direction, as anticipated. The other features are duplicated.

The investigation has included, also, portions of skin, from hard-shelled turtles and tortoises, alligators, lizards, and

snakes. When contrasted to these, however, the material described is found to be unequaled in its clear-cut demonstration of the relation of connective tissues, collagenic and elastic, to epidermis. Further, the lattice-work strata and intervening median septum of the Trionyx muticus provide a most satisfactory field for contrast study of dense and loose connective tissues.